MYCOTAXON

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MYCOTAXON

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EDITOR-IN-CHIEF

LORELEI L. NORVELL

editor@mycotaxon.com

Pacific Northwest Mycology Service
6720 NW Skyline Boulevard
Portland, Oregon 97229-1309 USA

NOMENCLATURE EDITOR

SHAUN R. PENNYCOOK

PennycookS@LandcareResearch.co.nz

Manaaki Whenua Landcare Research
Auckland, New Zealand

MYCOTAXON

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Reviewers — volume one hundred thirty-seven (4)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this issue.

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From the Editors

After nearly 50 years of publication, Mycotaxon is shutting its doors, probably forever. As the child that followed in his father's professorial footsteps, I felt it was most appropriate for me to have the honor of saying a few words about the history of Mycotaxon, and what Mycotaxon has meant to our family behind the scenes.

Growing up, I don't think any of us really understood what Mycotaxon was. Of course we knew that scientists from all over the world read the journal. One of the regular family activities was individually boxing and labeling the latest volume for shipping. The strange, foreign names were sometimes shouted out for their novelty or because we recognized them from previous boxing parties. I think we had a vague understanding that the contents were about naming mushrooms, but we had no idea what place Mycotaxon had in the science world at large.

My science roots are in the 1990s, a time when there was great passion for open source software and free data. We wanted to make everything freely available so that all scientists everywhere could benefit from our efforts. It was only then that I understood that Mycotaxon had been championing a similar cause for decades. Mycotaxon was born from the frustration that publication was both too expensive and too slow. One of the main inventions was requiring authors to provide camera-ready copy for photo-offset lithography. This radical idea saved money and time for both the journal and authors, meaning that publishing in Mycotaxon was cheaper, easier, and faster than other journals. Mycotaxon also required authors to suggest an arm's length reviewer, which not only saved editorial time, but was a predecessor to the current efforts in credit-for-review. Mycotaxon was way ahead of its time and I'm proud that my father was at the forefront of scientific publishing.

Mycotaxon has always been a family business. One critical and beloved member of the Mycotaxon family was Lorelei Norvell, who was Editor in Chief for 20 years. Lorelei recently passed. You will find an article about her in this issue. Finding someone with Lorelei's passion and attention to detail is a near impossible task, which is one of the reasons Mycotaxon is shutting its doors. Another reason is that Mycotaxon's job is largely done. It made publication easier and more accessible at a time when publication was difficult. It's no longer needed. Is there a new frontier where mycological taxonomy needs a shake-up? If so, maybe Mycotaxon will dust off its trousers and lead the charge. But until then, it's going to take a long-deserved holiday.

Ian F. Korf
Professor, Molecular and Cellular Biology
University of California at Davis

MYCOTAXON 137(4) presents 31 contributions by over 100 authors in institutions representing 22 countries. Within these articles are descriptions of 5 new genera (*Acropleurophialis; Apicheirospora; Neochrosporium; Owingsia; Porosynnema*), 26 new species, and 3 new combinations (*Lichenomphalia oniscus; Owingsia umbellifera; Steccherinum laxum*). Over 40 reviewers contributed their time to review the articles in this issue. We also present 3 new checklists that are hosted on our mycobiota page, including a checklist from a foray held a few months ago honoring one of the founders of this journal, Richard P. Korf. This issue of *Mycotaxon* is dedicated to Dr. Lorelei L. Norvell. The final article in this issue, written by three of her colleagues, celebrates her contributions to the field.

Regrettably, this issue ends the publication of *Mycotaxon*.

I want to thank Shaun Pennycook for his support during the last few months as together we assembled this final issue. Dr. Norvell had laid the groundwork for the issue, communicating with authors and approving their texts, but the finishing up of the issue was something I could only have accomplished with Shaun's assitance. I'd also like to thank the authors, reviewers, and our subscribers, for their patience, this issue is being published a year later than originally planned.

For the last 49 years, the last step in preparing an issue for publication was creating the cover. Our last editor, the late Dr. Lorelei Norvell, was unable to complete this issue, and did not indicate what images she would have chosen for the cover. When I took over this final issue, I had noted several candidates, from images submitted with the articles, but in a break with tradition, I chose to pursue a different idea for the cover images.

The covers for this issue are a history of *Mycotaxon*, by year, and by color. This data visualization highlights an unusual characteristic of the journal. One of the first decisions made by Grégoire and my father was that a volume of *Mycotaxon* would contain a standard minimum number of pages. Subscribers were guaranteed a certain number of pages (256+ at first, and later 512+) for their subscription. Once that number of pages had been completed, the volume would close, and another volume would begin, no matter where in the calendar year this fell. This allowed great flexibility in allowing the editors to fill a volume with current submissions, neither needing to "hold back" articles for a future issue, nor worrying that a particular issue would be either too thin or too thick. The importance for the editors of *Mycotaxon* was to get the articles out quickly and to make sure that subscribers got what they paid for. The flexibility extended to authors as well; because publishing in *Mycotaxon* was free* to authors, they were not constrained in the number of pages they could afford to write.

This variability in the size of the quarterly issue led to all manner of problems: the United States Post Office refused to recognize our mailings as a "normal" quarterly magazine and we therefore paid unusually high shipping rates, and for our institutional subscribers, who had likely never encountered such an odd approach to a subscription, innumberable billing headaches.

Since the early days, the volumes were distinguished by color. In the image below you can see that vol 31 had two numbers, volume 32 and 33 had just one, and volumes 34, 35, and 36 each had two numbers. Yet each of these took up the same amount of shelf space...literally, the same volume.

an example set of 8 volumes published from 1988 through 1990

After 30 years of this non-calendrical publishing, in mid-2014 Lorelei very reasonably suggested that since we had essentially been publishing 4 volumes a year for a good number of years, that we simply move to accepting annual subscriptions, and so from volume 130 onward, a volume was understood to be 4 quarterly issues, regardless of the number of pages.

For the first few decades, the journal was only printed in black ink, the covers were the Table of Contents, and the only cover colors available to us were those offered by the printer, but as we moved from offset to digital printing, we were able to print full-color photographs within the issue, and our editor made full use of the spectrum of colors available to her for the covers.

To create the data visualization, I looked at all the issues of *Mycotaxon* and captured their colors, mapping them to the year of its publication. The first row of data is a photograph of a shelf of the first five years of the journal held

1974	1975	1976	1977	1978
1979	1980	1981	1982	1983
1984	1985	1986	1987	1988
1989	1990	1991	1992	1993
1994	1995	1996	1997	1998

1999	2000	2001	2002	2003
2004	2005	2006	2007	2008
2009	2010	2011	2012	2013
2014	2015	2016	2017	2018
2019	2020	2021	2022	2023

maps of the front and back cover of this issue

at the Cornell University Plant Pathology Herbarium (CUP). The following 4 rows show subsequent sets of 5-year periods, with the color we chose to use for each of the volumes. As you can see, the color choices do not break neatly across the years. The colors also appear to be fairly random, with the exception of volume 25, which was silver, and volume 50, which was gold. Starting with volume 100 (April–June 2007), the cover of the journal was an image, chosen by Lorelei, rather than the table of contents. From volume 100 to 130(3) these images were pulled from the drawings authors submitted. The beauty of these line drawings is lost in the miniature versions, so in the visualization across the years, I only show a few, starting with volume 129(1) when we started assigning quarterly numbering. Beginning with volume 130(4), Lorelei started using photographs on the cover instead of line drawings.

On the back cover, the data display continues, covering the years 1999-2023**. You will notice that over time the colors chosen were no longer random, and each year/ each volume, had a theme color.

Lorelei took great care in choosing the cover images, often rendering several choices and sharing them with the other editors for feedback. She was an artist and a scientist, and her love for both is clear in the

care she took in designing the journal and creating the covers. I encourage readers to review her editorial notes for each issue. We always include the front and back covers as part of the editor's notes.

5 rows and 5 columns on the front, and 25 more on the back. 50 years. I'm sad to end the journal's publication. In 2024 I will be working to make all of the past articles available and open to all.

<div align="right">

Noni Korf
Manager, *Mycotaxon*
7 November 2023

</div>

*for all but a few years, there were no charges to authors to publish in *Mycotaxon*. There were two exceptions. First, color pages. In the early days an author had to pay extra for full-color images; these plates would be tipped-in to the otherwise black&white journal. And secondly, authors who wanted their articles to be Open Access at the time of publishing paid a fee for this. All the articles in this last issue are Open Access (at no charge to authors) and very soon the entire catalog will be available to readers.

**Note that though this last issue is being published in 2023, it is part of the 2022 quarterly publication.

Errata

Volume 134(2), page 720

in article "Notes on rust fungi in China 7. *Aecidium caulophylli* life cycle inferred from phylogenetic evidence and renamed as *Puccinia caulophylli* comb. nov." the authors, Jing-Xin Ji, Zhuang Li, Yu Li, and Makoto Kakishima, request that the final column in Table 1 be replaced with the correct data below, highlighted in yellow.

Table 1. Sequence data analyzed in this study

Species	Host plant	Locality in China	Date	Voucher Specimen	GenBank accession No. ITS	GenBank accession No. 28S
Puccinia caulophylli	*Caulophyllum robustum*	Hongyegu, Jiaohe, Jilin, Jilin	24-Jun-2015	HMJAU8531	MK785259	MK785284
	C. robustum	Lafa Mountain, Jiaohe, Jilin, Jilin	23-Jun-2015	HMJAU8532	MK785260	MK785285
	C. robustum	Bangchuigu, Jiaohe, Jilin, Jilin	1-Jul-2018	HMJAU8624	MK785261	MK785286
	C. robustum	Qingling, Jiaohe, Jilin, Jilin	29-Jun-2017	HMJAU8620	MK785262	MK785287
	C. robustum	Hongyegu, Jiaohe, Jilin, Jilin	24-Jun-2015	HMJAU8534	MK785273	MK785298
	Milium effusum	Fenghuang Mountain, Wuchang, Heilongjiang	5-Jul-2016	HMJAU8619	MK785271	MK785296
	M. effusum	Changbai Mountain, Erdaobaihe, Yanbian, Jilin	28-Jul-2015	HMJAU8618	MK785272	MK785297
	M. effusum	Lushuihe Forest Park, Baishan, Jilin	2-Sep-2018	HMJAU8625	MK785274	MK785299
	M. effusum	Bangchuigu, Jiaohe, Jilin, Jilin	12-Sep-2017	HMJAU8622	MK785275	MK785300
	M. effusum	Erdaobaihe, Yanbian, Jilin	2-Sep-2018	HMJAU8627	MK785276	MK785301
	M. effusum	Lafa Mountain, Jiaohe, Jilin, Jilin	1-Jul-2018	HMJAU8623	MK785277	MK785302
	M. effusum	Lushuihe Forest Park, Baishan, Jilin	3-Sep-2018	HMJAU8626	MK785278	MK785303
	M. effusum	Fenghuang Mountain, Wuchang, Heilongjiang	9-Sep-2017	HMJAU8621	MK785279	MK785304
Puccinia adenocauli	*Carex onoei*	Qingling, Jiaohe, Jilin, Jilin	12-May-2018	HMJAU8630	MK785267	MK785292
	C. onoei	Jilin Agricultural University, Changchun, Jilin	2-Jul-2017	HMJAU8274	MK785268	MK785293
	Adenocaulon himalaicum	Bangchuigu, Jiaohe, Jilin, Jilin	1-Jul-2018	HMJAU8629	MK785269	MK785294
	A. himalaicum	Fenghuang Mountain, Wuchang, Heilongjiang	23-Jun-2017	HMJAU8628	MK785270	MK785295
Puccinia klukistianum	*Ligustrum obtusifolium*	Jilin Agricultural University, Changchun, Jilin	24-Jun-2018	HMJAU8633	MK785263	MK785288
	L. obtusifolium	Jilin Agricultural University, Changchun, Jilin	12-Jun-2015	HMJAU8198	MK785264	MK785289
	L. obtusifolium	Jingyuetan Forest Park, Changchun, Jilin	25-Jun-2017	HMJAU8631	MK785266	MK785291
	Cleistogenes hackelii	Jilin Agricultural University, Changchun, Jilin	28-Aug-2016	HMJAU8282	MK785265	MK785290
	C. hackelii	Jilin Agricultural University, Changchun, Jilin	9-Jul-2016	HMJAU8280	MK785280	MK785305
	C. hackelii	Jingyuetan Forest Park, Changchun, Jilin	16-Sep-2017	HMJAU8632	MK785281	MK785306
Gymnosporangium yamadae	*Malus baccata*	Jilin Agricultural University, Changchun, Jilin	10-Sep-2014	HMJAU8096	MK785282	MK785307
Gymnosporangium asiaticum	*Pyrus* sp.	Wunvfeng Forest Park, Jian, Tonghua, Jilin	8-Jun-2016	HMJAU8324	MK785283	MK785308

Buellia parmigera sp. nov. from China

Min Ai [1,2], Xin-Yu Wang [1,2], Fiona Ruth Worthy[1,2], Li-Song Wang [1,2*]

[1] *Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, CAS;*
   Kunming 650000, Yunnan, China

[2] *Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, CAS;*
   Kunming 650000, Yunnan, China

* Correspondence to: *wanglisong@mail.kib.ac.cn*

Abstract—A new species, *Buellia parmigera,* was discovered from the Tibetan plateau, characterized by a crustose-subsquamulose thallus, immersed and lecanorine apothecia, a hyaline hypothecium, and *Buellia*-type ascospores. The new species is described, and compared with the other *Buellia* species with lecanorine apothecia.

Key words—lichenized fungi, *Ascomycota*, taxonomy, *Caliciaceae*

Introduction

Buellia De Not. (*Caliciales, Caliciaceae*) was introduced by De Notaris (1864), and Müller & von Arx (1962) designated *Buellia disciformis* (Fr.) Mudd as its type species. *Buellia* is characterized by a crustose thallus, black lecideine apothecia, *Bacidia*-type asci, brown ascospores with one or more septa, and a reddish-brown or rarely hyaline hypothecium. Since its establishment, approximately 400 species have been placed in the genus (Bungartz & al. 2007). Several other genera previously included in *Buellia* s.lat. (e.g., *Amandinea* Scheid. & H. Mayrhofer, *Diplotomma* Flot., and *Tetramelas* Norman) have since been segregated based on their macro- and micromorphology, chemistry, and ecological environment (Scheidegger 1993, Marbach 2000, Nordin 2000). However, due to the lack of phylogenetic studies, there remain many species

currently placed in *Buellia* s.lat. that may eventually be assigned to other genera. Over 64 species of *Buellia* s.lat. have been reported from China; these were mostly collected within the Tibetan Plateau region (Wei 2020, Wang & al. 2020). During the Second Tibetan Plateau Scientific Expedition and Research Program (STEP), more than 1200 specimens of *Buellia* were collected. Based on examination of morphology, coupled with chemistry and phylogenetic analyses, we propose *Buellia parmigera* as a new species. In this paper, we present a phylogenetic study of *Caliciaceae*, based on a nrITS matrix. There is no evidence that would support this new species being assigned to any genus separate from *Buellia* s.lat., so we have treated it as belonging to the broad concept of *Buellia*. Detailed descriptions and figures for the proposed new species are also provided.

Materials & methods

Morphological and chemical analyses

Specimens used in this study were collected from the Hengduan Mountains area and the Qinghai-Tibet plateau. They were all deposited in the Lichen Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN). Macromorphological studies were conducted under a dissecting microscope (Nikon SMZ 745T). Micromorphology was examined under an optical microscope (Nikon Eclipse Ci-S). Photographs were taken using a digital camera (Nikon DS-Fi2). The lichen secondary metabolites were detected and identified by spot tests [K (10% KOH), C (Ca(OCl)$_2$), KC, PD (P-phenylenediamine ethanol saturated liquid)] and thin-layer chromatography, using solvent systems C (toluene : acetic acid = 85 : 15) (Orange & al. 2001).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh materials using DNA secure Plant Kits (TIANGEN), according to the manufacturer's instructions. The primers were ITS1F & ITS4 (White & al. 1990, Gardes & Bruns 1993). PCR amplification reactions were performed in a 25 μL volume, containing 3 μL of genomic DNA, 1 μL of a 10 mM solution for each primer and 20 μL of 1.1 × T3 Super PCR Mix (TSINGKE). The PCR profile for the target region was as follows: initial denaturation at 98°C for 3 min, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 54–56°C for 10 s, elongation at 72°C for 15 s; and a final extension at 72°C for 2 min. PCR product sequencing was carried out with the same amplification primers, using Sanger technology by Tsingke Biotechnology Co., Ltd. (Kunming, China).

Phylogenetic analyses

Sixty-eight samples representing *Caliciaceae* Chevall. and *Physciaceae* Zahlbr. were downloaded from GenBank and eight samples of *Caliciaceae* were obtained from our materials. All sequences were edited manually using Geneious v8.0.2. The nrITS matrix was aligned using MAFFT v7 with the option of E-INS-I (Katoh & al. 2005). Ambiguous regions were excluded using Gblocks (Talavera & Castresana 2007) with the default settings. Phylogenetic relationships were inferred based on Bayesian inference (BI) and maximum likelihood (ML).

ML analyses were performed with RAxML v8.2.12 (Stamatakis 2006) with 2000 rapid bootstrap replicates. The best-fit partition substitution models were selected based on the lowest Bayesian information criterion (BIC) using Partition Finder 2 (Guindon & al. 2010, Lanfear & al. 2012, 2017): TIM2e+G4. Bootstrap support values (BS) were estimated from the 70% majority rule tree from all saved trees. BI analyses were performed with MrBayes v3.2.7 (Ronquist & al. 2012), running for 2 million generations. The trees were sampled every 100 generations and the first 25% of the trees were discarded as burn-in. Bayesian posterior probabilities (PP) were obtained from the 95% majority rule consensus tree of all saved trees. FigTree v1.4.0 (Rambaut 2012) and Adobe Illustrator were used to view and edit the phylogenetic tree.

Results

The nrITS matrix comprised 76 sequences including eight newly generated sequences (Table 1). Eight representative monophyletic genera were selected from *Caliciaceae* and several *Physciaceae* species were selected as the outgroup. The BI and ML trees showed similar topologies. Therefore, only the ML tree has been provided (Fig. 1). The results of the phylogenetic analyses showed that the specimens designated as *Buellia parmigera* were clustered within *Caliciaceae* with strong statistical support (BS = 96%; PP = 1) and they formed a highly supported monophyletic lineage (BS = 100%; PP = 1). There is no evidence that *B. parmigera* belongs to any genus separate from *Buellia* s.lat., so it is retained there. *Buellia parmigera* was sister to *B. aethalea* (Ach.) Th. Fr. with high support (BS = 95%). In addition, the clade including *B. parmigera* and *B. aethalea* was sister to the clade of *B. chujana* Xin Y. Wang & al. and *B. badia* (Fr.) A. Massal. with good support (BS = 91%; PP = 0.96). There were distinctively different micromorphological characteristics for *B. parmigera* versus other species in *Buellia* s.lat. We therefore recognize *B. parmigera* as a new species.

TABLE 1. Species, specimens, and sequences used in the phylogenetic analyses. Newly obtained sequences are in **bold** font.

TAXON	VOUCHER	GENBANK (ITS)
Acolium inquinans	Wedin 6352 (UPS)	AY450583
A. karelicum	Hermansson 16472 (UPS)	KX512897
Amandinea lignicola	Toensberg 36426 (BG)	JX878521
A. punctata 1	AFTOL 1306	HQ650627.1
A. punctata 2	Nordin 5346	AF224353
A. punctata 3	000272563 (GZU)	GU553286
Anaptychia ciliaris	Wedin 6429 (UPS)	AY143391
Buellia aethalea 1	Per Johansson 5 (UPS)	AF540496
B. aethalea 2	F138222 (S)	JX000098
B. badia 1	Westberg 09-079 (S)	KX512900
B. badia 2	TS1767 (LCU)	MG250192
B. chujana 1	140835-1	KT733597
B. chujana 2	140835-2	KT733598
B. disciformis	Nordin 4429 (UPS)	AF540498
B. erubescens 1	Wetmore 95879 (S)	KX512902
B. erubescens 2	CBM: Watanuki: L01004	LC069373
B. erubescens 3	CBM: Watanuki: L01032	LC069374
B. erubescens 4	KW 63381	GU553289
B. griseovirens 1	Nordin 4734 (UPS)	AF540500
B. griseovirens 2	Lendemer 28500 (NY)	KC681819
B. griseovirens 3	Lendemer 28474 (NY)	KC681820
B. muriformis	Nordin5336a (UPS)	AF540501
B. numerosa 1	CBM: Watanuki: L01033	LC153798
B. numerosa 2	CBM: Watanuki: L01034	LC153799
B. parmigera	XY20-129 (KUN)	**ON166694**
B. parmigera	XY20-268 (KUN)	**ON166695**
B. parmigera	XY20-176 (KUN)	**ON166696**
B. parmigera	XY20-272 (KUN)	**ON166701**
B. parmigera	XY20-274 (KUN)	**ON166697**
B. parmigera	XY20-292 (KUN)	**ON166698**
B. parmigera	XY20-276 (KUN)	**ON166699**
B. parmigera	XY20-275 (KUN)	**ON166700**
B. penichra	Nordin5322 (UPS)	AF540503
B. subnumerosa 1	CBM: Watanuki: L01049	LC153800
B. subnumerosa 2	CBM: Watanuki: L01843	LC153803
B. subnumerosa 3	CBM: Watanuki: L01423	LC153801
Calicium nobile 1	Tibell 21968 (UPS)	KX512913

Taxon	Voucher	GenBank (ITS)
C. nobile 2	Tibell 23396 (UPS)	KX512914
Diplotomma alboatrum 1	18-60034 (KUN)	MN615696
D. alboatrum 2	18-60448 (KUN)	MZ224658
D. alboatrum 3	Prieto 3034 (S)	KX512924
D. alboatrum 4	Uppland, 2001 Nordin	AF408677
D. alboatrum 5	Nordin 5345	AF224351
D. alboatrum 6	Nordin 3205 (UPS)	DQ198357
D. pharcidium 1	—	FR799180
D. pharcidium 2	—	FR799314
D. venustum 1	18-58557 (KUN)	OL467349
D. venustum 2	18-58102 (KUN)	OL467350
D. venustum 3	XY19-252 (KUN)	OL467353
Heterodermia speciosa	Wetmore (S)	KX512927
H. vulgaris	Frisch 11/Ug1226 (UPS)	KX512928
Phaeophyscia ciliata	Prieto (S)	KX512929
Ph. orbicularis	Prieto 3012 (S)	KX512930
Physcia aipolia	Wedin 6145 (UPS)	KX512931
P. tenella	Odelvik & Hellström 0827 (S)	KX512932
Pseudothelomma ocellatum 1	Tehler 8063 (S)	KX512934
Pse. ocellatum 2	Hermansson 18662 (UPS)	KX512935
Pyxine cocoes	Prieto (S)	KX512936
Py. endochrysina 1	14-46462 (KUN)	KY611887
Py. endochrysina 2	14-46439 (KUN)	KY611888
Py. sorediata	Wetmore 91254 (S)	KX512937
Py. subcinerea	—	HQ650705
Rinodina cinnamomea 1	Spribille 19893 (GZU)	KX015688
R. cinnamomea 2	Spribille 20101 (GZU)	KX015689
R. degeliana 1	Tonsberg 41921	KX015674
R. degeliana 2	Thor 28169	KX015675
Tetramelas chloroleucus	Westberg 10–001 (S)	KX512938
T. geophilus	Nordin 4429 (UPS)	AF540499
T. insignis 1	Nordin 5664 (UPS)	DQ198358
T. insignis 2	ZT2013043	KP314327
T. pulverulentus	Nordin 6368 (UPS)	KX512940
T. triphragmoides	Nordin 4425 (UPS)	AF540505
Thelomma mammosum 1	Tibell 23775 (UPS)	KX512942
T. mammosum 2	Hernández et al. 2002 (UPS)	KX512943
T. santessonii 1	Nordin 4011 (UPS)	KX512944
T. santessonii 2	Nash 38262 (UPS)	KX512945
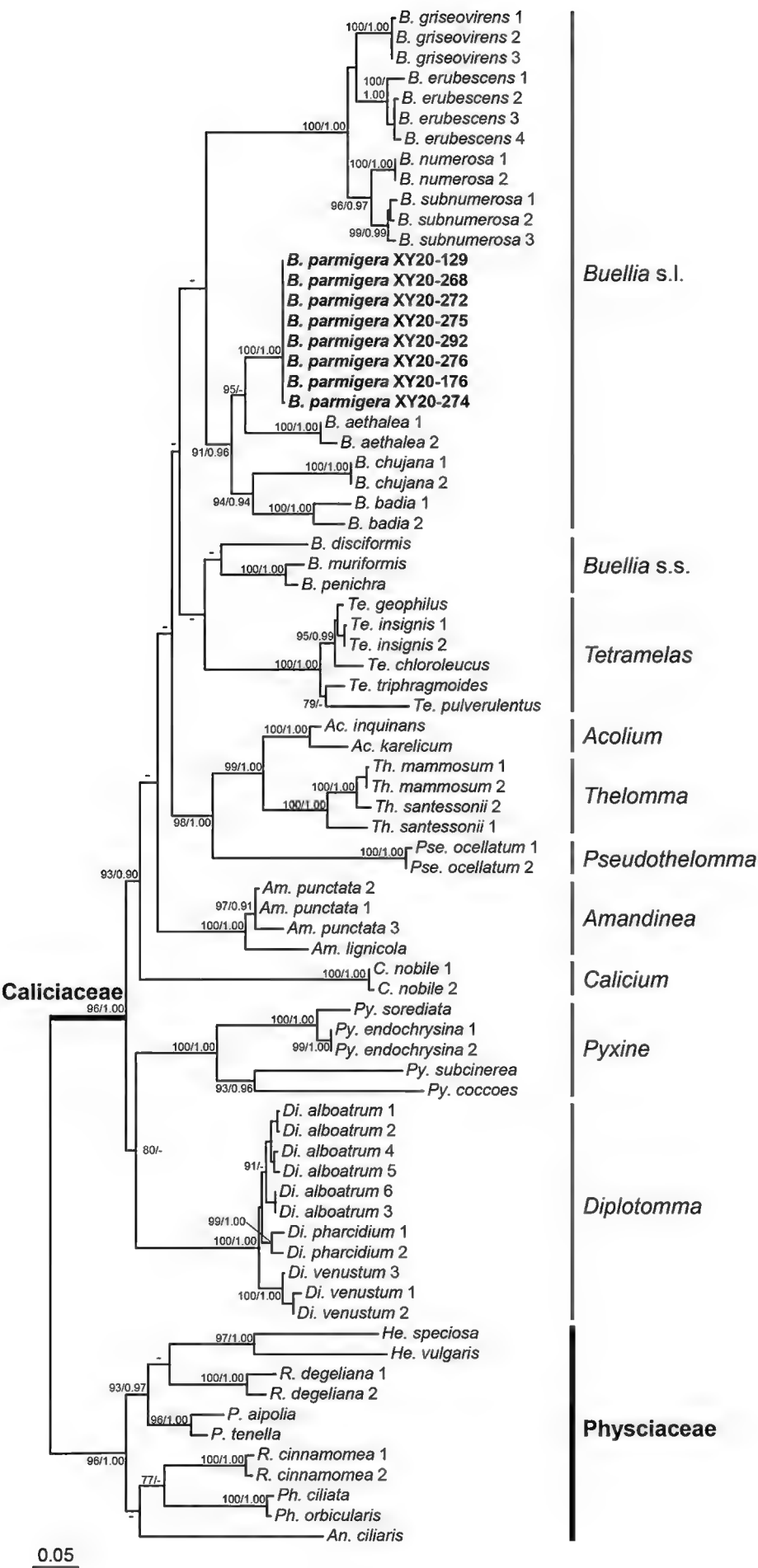
FIG. 1. RAxML tree of *Caliciaceae* based on analysis of nrITS region. Bootstrap support values for Maximum likelihood ≥ 75% and Bayesian posterior probabilities ≥ 0.90 are indicated near the nodes. The new species is shown in **bold**.

Taxonomy

Buellia parmigera M. Ai & Xin Y. Wang, **sp. nov.**   Fig. 2.
MB 843667

Differs from other Chinese *Buellia* species by its lecanorine apothecia and hyaline hypothecia; and from *Rinodina* spp. by its *Bacidia*-type asci and *Buellia*-type ascospores.

Type—China, Sichuan Province, Liangshan Yi Autonomous Prefecture, Yanyuan County, Weicheng town, 27.5015°N 101.6864°E, alt. 2814 m, on rock, 28 Jul. 2020, X.Y. Wang & al. XY20-268 (**Holotype**, KUN).

Etymology—*parmigera* (Latin = shield-bearer) refers to the shieldlike appearance of each areola and its single central apothecium, within the subsquamulose thallus.

Thallus crustose, areolate, closely adnate, up to 2 cm in diam., areolae irregular in shape, up to 0.5 mm in diam., sometimes subsquamulose, prothallus absent; upper surface yellow-brown to greenish brown, dull, without pruina; medulla white, non-amyloid (I–). Apothecia dense, usually aggregate in the central part, 1 per areola, lecanorine, immersed, disc dark brown to black , margin concolorous with the thallus, roundish to irregular, 0.1–0.5 mm in diam., margin persistent; thalline exciple lecanorine-type, algal layer continuous under the hypothecium; epihymenium yellowish brown to brown; hymenium hyaline, 50–60 μm tall, not inspersed, paraphyses simple to moderately branched, apically swollen, with a brown pigment cap; hypothecium hyaline, c. 40 μm tall; asci oval-clavate, *Bacidia*-type, 8-spored, spores 1-septate, hyaline when young, turning brown when mature, *Buellia*-type (Bungartz & al. 2007), ellipsoid, with obtuse ends, proper septum narrow, thickened when young, (9–)10–12(–13) × (4–)5–6(–7) μm. Pycnidia not seen.

Chemistry—Thallus K–, C–, KC+ pink, PD–, UV–, medulla I–; containing gyrophoric acid.

Distribution & ecology—This new species is mainly distributed in the Hengduan Mountains area, growing on exposed siliceous rock at elevations of (1200–)2500–3500 m.

Additional specimens examined (vouchered in KUN)—CHINA, Yunnan Province, **Shangri-la City**, Jiantang town, Potatso National Park, 27.8280°N 99.9784°E, alt. 3500 m, on rock, 25 Jul. 2020, X.Y. Wang & al. XY20-129; **Lijiang City**, Ninglang County, Xichuan Village, 27.0938°N 100.6070°E, alt. 2517 m, on rock, 26 Jul. 2020, X.Y. Wang & al. XY20-176. Sichuan Province, **Liangshan Yi Autonomous Prefecture**, Yanyuan County, Weicheng town, 27.5015°N 101.6864°E, alt. 2819 m, on rock, 28 Jul. 2020, X.Y. Wang & al. XY20-275, XY20-292; **Dechang County**, Leyue Town, 27.3190°N 102.3273°E, alt. 1268 m, on rock, 29 Jul. 2020, X.Y. Wang & al. XY20-353. Xizang Province, **Xiayadong Village**, 27.4313°N 88.9125°E, alt. 3187 m, on rock, 26 Jul. 2019, X.Y. Wang & al. XY19-2495.
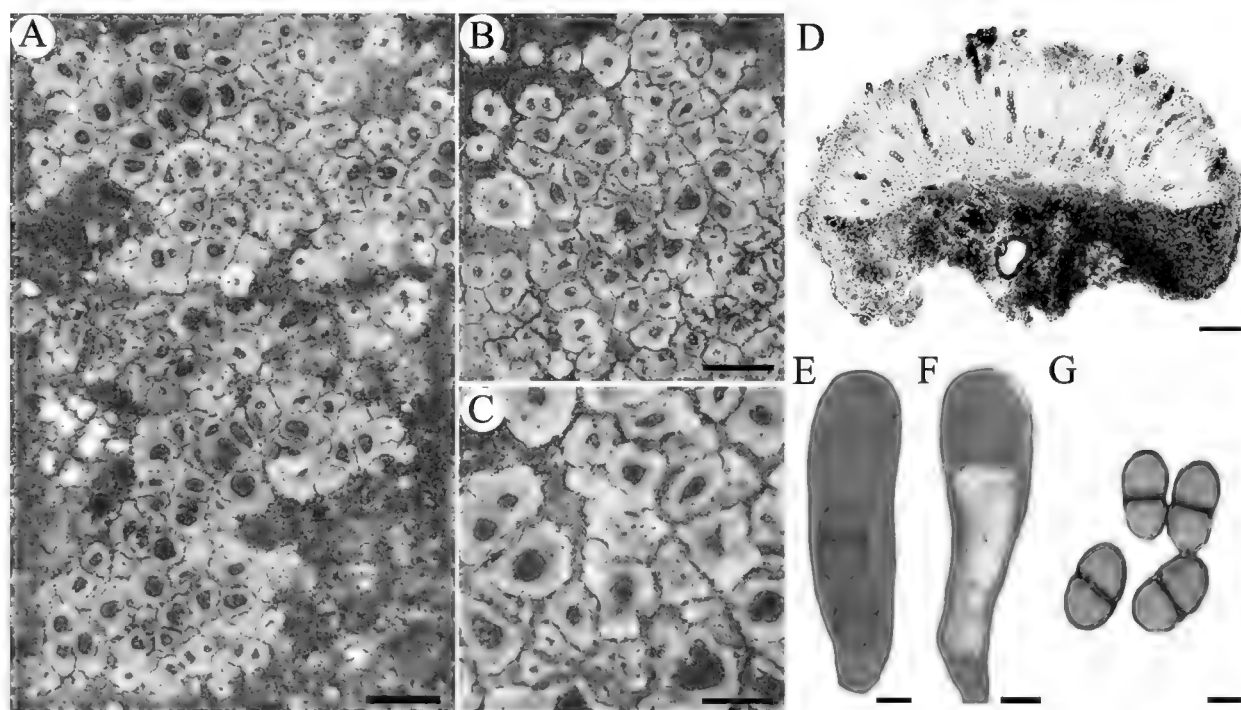
FIG. 2. *Buellia parmigera* (holotype, KUN–XY20-268). A–C. Subsquamulose thallus and immersed apothecia; D. The section of apothecium, showing lecanorine-type exciple and hyaline hypothecium; E, F. *Bacidia*-type asci; G. Ascospores, *Buellia*-type, 1-septate. Scale bars: A, B = 1 mm; C = 0.5 mm; D = 50 μm; E–G = 5 μm.

NOTES—This new species could be distinguished from all other Chinese *Buellia* species by its saxicolous thallus, lecanorine apothecia, hyaline hypothecium and the presence of gyrophoric acid. It could potentially be misidentified as a *Rinodina* species due to its lecanorine apothecia and hyaline hypothecium, but it is confirmed as belonging to *Buellia* according to phylogenetic study and ascospore ontogeny.

Some *Buellia* species also have lecanorine apothecia at least during the early stage of development, such as *B. dakotensis* (H. Magn.) Bungartz, *B. endoferruginea* Bungartz, and *B. fouquieriensis* Bungartz. All these species have brown to reddish brown hypothecium and lecanorine apothecia which become lecideine when mature, and have a corticolous habit (Bungartz & al. 2007). The saxicolous species *Buellia mamillana* (Tuck.) W.A. Weber has lecanorine apothecia when young, but differs by having a pale greenish yellow upper surface, *mamillana*-type apothecia and the presence of xanthones (Bungartz & al. 2004).

Another species of *Buellia* containing only gyrophoric acid is *Buellia uberior* Anzi, with a wide distribution across the Northern Hemisphere (Bungartz & al. 2007), but it differs by having lecideine apothecia, deep reddish brown hypothecium, and epihymenium with cinereorufa-green pigments (HNO_3+ violet).

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MYCOTAXON

Lichenomphalia umbellifera: fungible and infungible epithets and species concepts

ANDRUS VOITK¹*, GREG THORN², IRJA SAAR³

¹ *Foray Newfoundland and Labrador, 13 Maple St, Humber Village*
  Newfoundland and Labrador, A2H 2N2, Canada

² *Department of Biology, University of Western Ontario,*
  London, Ontario, N6A 5B7, Canada

³ *Institute of Ecology and Earth Sciences, University of Tartu,*
  Juhan Liivi 2, 50409 Tartu, Estonia

*CORRESPONDENCE TO: minaise@nf.sympatico.ca

ABSTRACT—Comparison of the protologue of *Agaricus umbellifer* L. with specimens and descriptions of the basidiolichen *Lichenomphalia umbellifera* (L.) Redhead & al. revealed that the epithet *umbellifera* was misapplied to the *Lichenomphalia* species, causing several major conflicts with Linnaeus's species concept. A felicitous match for Linnaeus's species concept was found with a species of *Marasmius* sect. *Epiphylli*. Because *A. umbellifer* falls in a group that arises from an evolutionary pathway divergent from that leading to *Marasmius* s. str., we erected a new genus, *Owingsia*, to accommodate it, and recombined the type species as *Owingsia umbellifera*. Molecular studies demonstrated that it is a widely distributed circumpolar species, prevalent in Lapland and islands of the Baltic Sea, where Linnaeus encountered it. The earliest legitimate description of the basidiolichen *L. umbellifera* is *A. pseudoandrosaceus* Bull., a name superseded by the sanctioned later synonym, *A. ericetorum* Pers. We recombined this basionym as *L. ericetorum*, and epitypified *O. umbellifera* and *L. ericetorum* with modern sequenced specimens.

KEY WORDS—nomenclature, taxonomy

Introduction

> **How serious the conflict must be before a lectotypification can be superseded is a matter of opinion** . . . —JØRGENSEN & RYMAN, 1994

The nomenclatural type is not necessarily the most typical or representative element of the taxon. —THE SHENZHEN CODE, ART. 7.2

The basidiolichen currently known as *Lichenomphalia umbellifera* is distributed throughout the Northern Hemisphere (Geml & al. 2012). Because it prefers northern barrens over other habitats, it is ubiquitous in the Canadian province of Newfoundland and Labrador (NL), and the authors know it well: authors AV & GT have collected it in NL for some 20 years and author IS knows it from her native Estonia. AV can find it regularly about one hour's walk from his front door, on Mt. Ignoble, a hilltop laid barren by forest fire almost 100 years ago. Between 2003 and 2019, Foray Newfoundland & Labrador (FNL, the provincial mushroom club) recorded it 74 times on its annual forays, and AV made 32 collections of it at other times. The basidiolichen has a very variable macroscopic appearance (FIG. 1) regarding colour, shape, and gill arrangement and attachment; basidia with 1, 2, 3, and 4 sterigmata, producing spores smaller in size as the count goes up; diverse habitat tolerance, found from arctoalpine to lowland regions, in barrens, woodlands and even a grassy road embankment; and wide substrate preferences, most prevalent in peat or *Sphagnum* L., but also fruiting on bare ground with moss, on heath, bog, and characteristically on moss-covered fallen logs.

In the early years of surveying the funga of NL, we knew the current *L. umbellifera* as *Omphalina ericetorum*. By 2006 the newly combined *L. umbellifera* (Redhead & al. 2002) had become the only name used for it on FNL species lists. We applied first one, then the other name, without question. A need to review their protologues and nomenclatural history only arose after almost two decades, precipitated by a taxonomic review of some of its synonyms (Voitk 2022): a preliminary reading of the protologue suggested that the current application of the epithet might be at odds with the original material. This study was undertaken to investigate that question formally. To clearly differentiate between Linnaeus's *Agaricus umbellifer* and the basidiolichen currently known as *Lichenomphalia umbellifera*, in our discussions we represent the basidiolichen by the contraction AM-MIN, from "Amanita minima", the first two words Linnaeus (1732) used to describe it on his first encounter.

Species Fungorum (https://www.speciesfungorum.org/GSD/GSDspecies.asp?RecordID=375200; last accessioned 13 Mar 2022) provides a quick overview of the nomenclatural history of AM-MIN: a plethora of names have been applied to the species, the earliest being *Agaricus* "*umbelliferus*", introduced by Carl Linnaeus (1753). [In both Classical and Botanical Latin, the correct masculine adjective is *umbellifer*; and the orthographic variant "*umbelliferus*"
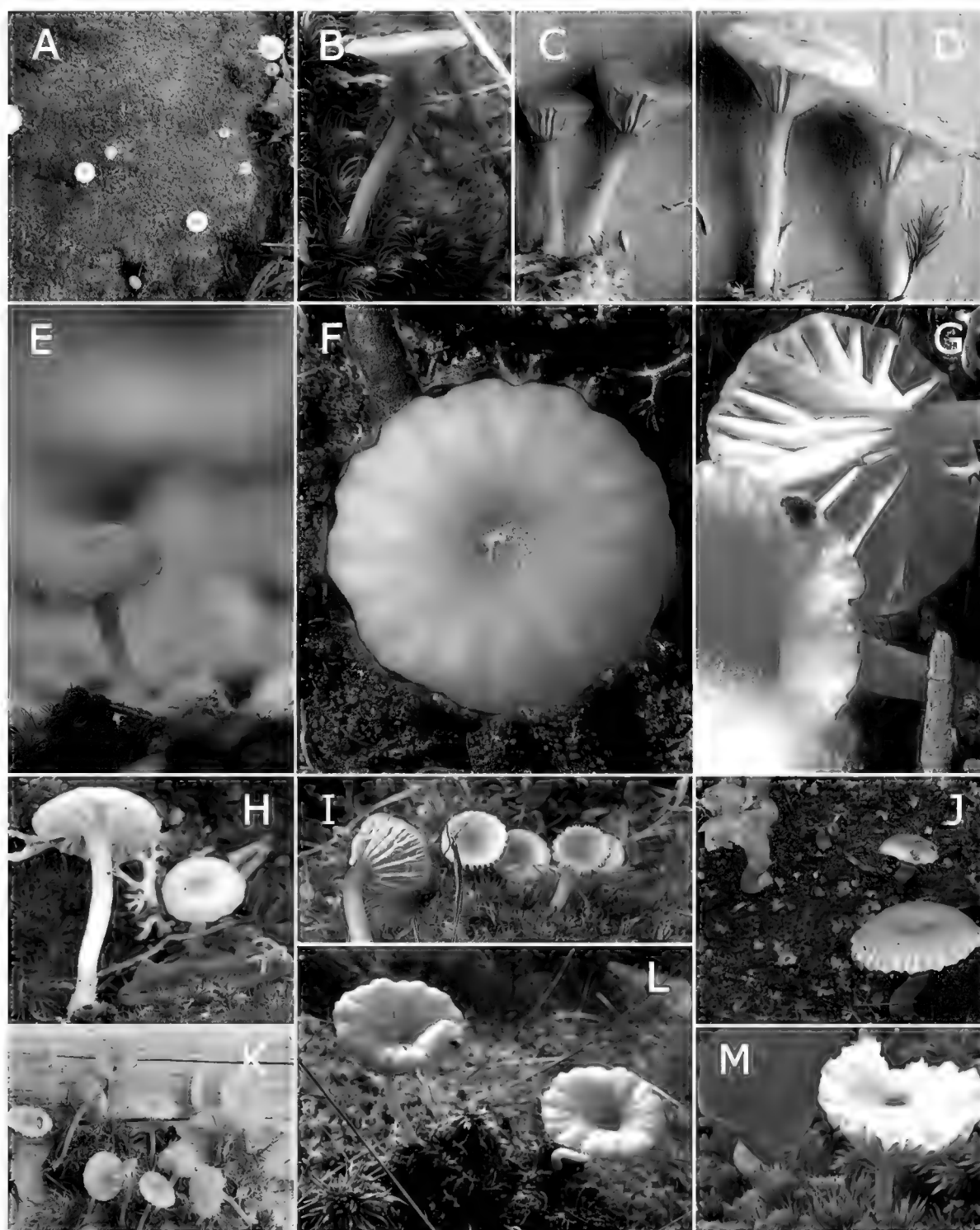
Fig. 1. Collage of several presentations of the mercurial AM-MIN in NL, also showing the fit with many descriptive names used over the years. Colour: from near-white (H, L, M), fitting with "niveus", to yellow (D, F, G), to tan-brown (I, K), to darker or duskier (fuscous) brown (B, C, E, J). At times purplish hues visible, characteristically on the upper stipe (B, C), fitting with "luteolilacina". Pileus near-plane (F, H, K), dome-shaped (E, F), upturned (G, I, L), or turbinate-obconical (C, D, M), minutely asperous (D, F, G). Central depression almost absent or shallow (F, H, K), narrow (C, M), or wide and deep, tapering to a sharp point (G, I, L). Lamellae adnate (H, K), to decurrent (C, D, G, L). Stipe most commonly sturdy (relatively wide), but on occasion delicate or narrow (A, K, L). Generally less than two cap diameters long, but occasionally reaches two cap diameters (I, L). Bends upwards on sides of peat pits (A, J), fitting with "turficola". Occasionally rises with a knock-kneed curve (L), fitting with "valgus". Usually bounteous botryoid lichen thallus readily visible at the base of the stipe (F, J), often covering neighbouring moss or sphagnum (C, D, L).

is correctable under ICN Art. 60.1 (Turland & al. 2018; henceforth the Code).] In total, Am-min has been referred to 19 genera, using 17 different specific epithets, and 21 different subspecific epithets, eight of which have not been used at the species level. These synonyms refer to a wide array of shapes (agaricoid, omphalinoid, umbelliferous, conical), colours (white, yellow, gray, pink, lilac), substrates (algae, turf, leaves, grass, wood), and other characters, with very little to support a uniform species concept. No doubt the mercurial nature of Am-min (Fig. 1) is partly responsible for some of these synonyms, but their profusion and in some cases seeming incompatibility suggests that other factors may be operational as well.

Our plan was to begin by establishing Linnaeus's concept of *Agaricus umbellifer*. Should a clear picture result, it can be tested for fit with Am-min. Should it fit, the name is well applied, and our quest is over. Should it not fit, we decided two tasks needed to be completed: 1) identify a fitting species to which *A. umbellifer* can be applied, and 2) find an acceptable name for Am-min.

Determining Linnaeus's species concept of *Agaricus umbellifer*

Method

All descriptions by Linnaeus were examined for uniform and consistent characters, to get an idea of his species concept for this name. The protologue (Linnaeus 1753) was assigned greatest authority. Blindly imposing conspecificity or synonymy on all cited descriptions by others produced illogical conflicts. For example, this approach led Redhead & Kuyper (1987) to conclude that Linnaeus (1753) included more than one species by citing a description and illustration by Micheli (1729) of a long-stemmed species with a bowl-shaped cap, while describing a flat-capped one. To claim that he cited a round-capped species as conspecific did not make sense, provided he considered cap shape an interspecific character. However, because Linnaeus did not state that he considered any of his citations conspecific or synonymous, we decided to avoid assigning an arbitrary significance to them that he may not have intended. Rather, we assumed that he selected the most accurate match he could find from a limited field of similar species in order to demonstrate major characters he considered important to *A. umbellifer*, but did not designate them as synonyms because he did not believe they were. With this approach, the citations made sense, and enabled us to use cited descriptions with some conflicting elements to note those shared characters specifically stressed by Linnaeus and other cited authors.

Linnaeus's travels to Lapland were in northern Fennoscandia (not in the modern Finnish political region of Lapland). Throughout this discussion we

interpret Lapland as the ethnocultural region Sápmi, traditionally occupied by the Sámi people, encompassing the northern parts of Norway, Sweden and Finland as well as the adjoining Kola Peninsula of Russia. Because the region has no politically defined borders, its exact extent varies with different descriptions; we used the area defined by Pinto-Guillaume (2017).

Results

Table 1 summarizes Linnaeus's efforts to circumscribe *Agaricus umbellifer* morphologically and ecologically. Characters of illustrated treatments are taken from both descriptions and illustrations. A character is marked present (+) only if listed or directly quoted in the work.

Linnaeus described two personal encounters with the species, the first in Lapland (Linnaeus 1737) with the phrase name *Agaricus caulescens albus parvus, petiole longo, pileo plano pellucido, margine multifidio*, and the second in Öland (Linnaeus 1741) with the phrase name *Agaricus minimus capitulo turbinato plano albo, lamellis margine fuscis*. We know he considered them conspecific because in the first edition of Flora Suecica (Linnaeus 1745) he quoted both these phrase names in full when describing the same entity as species #1033, with the phrase name *Agaricus caulescens, pileo plicato membranaceo lamellis basi latioribus*. As the only additional character, he added that it is found among decaying fallen leaves (inter semiputrida dejecta folia sylvarum). In addition to his own previous phrase names, Linnaeus (1745) quoted phrase names by Micheli (1729) and Ray (1724) in full, cited another by Haller (1742), as well as illustrations by Micheli and by Buxbaum (1733). In Species Plantarum (Linnaeus 1753), the work where he introduced use of binomial names, for species # 22, *A. umbellifer*, he quoted in full his phrase names from the Lapland, Öland, and the Swedish flora (Linnaeus 1737, 1741, 1745), as well as the phrase name by Micheli, again citing the latter's illustration. Under the modern Code, the starting date for valid fungal nomenclature has been set back to 1753, making the Species Plantarum description the nomenclatural protologue for *A. umbellifer*. As before, the only descriptive information he added was to repeat that the species occurred among piles of decaying leaves. Linnaeus's final treatment of the species came two years later in the second edition of Swedish flora (Linnaeus 1755) as species #1192. Apart from the change from the phrase name used in the first edition to the binomial, the description repeated that of the first edition.

TABLE 1. Linnaeus's species concept of Agaricus umbellifer, in comparison with the neotype of Owingsia umbellifera

	BASIDIOMA		PILEUS			STIPE	LAMELLAE		SUBSTRATE
	WHITE	TINY	FLAT	PLICATE	STRIATE	LONG	DISTANT	BROAD	LEAFY
Linnaeus 1737	+	+	+	+	+	+	–	–	–
Linnaeus 1741	+	+	+	+	–	–	–	–	–
Linnaeus 1745	+	+	+	+	+	+	+	+	+
Linnaeus 1753 protologue	+	+	+	+	+	+	+	+	+
Linnaeus 1755	+	+	+	+	+	+	+	+	+
Ray 1724	+	+	–	–	–	+	–	–	–
Micheli 1729	+	+	–	–	+	+	+	–	+
Buxbaum 1733	+	+	–	+	–	+	–	–	–
Haller 1742	+	+	+	–	–	+	–	–	+
Total	9	9	6	6	5	8	4	3	5
Owingsia umbellifera neotype	+	+	+	+	+	+	+	+	+

The sum of Linnaeus's descriptions and citations gel into a lucid concept (TABLE 1): *A. umbellifer* is a small, white mushroom with a flat, somewhat translucent, radially segmented cap, distant adnate gills, a long stem, growing on fallen leaves. A few characters deserve comment. Pellucid, membranaceous, and striate are interpreted as different ways to indicate a translucent cap with visible radial lamellar projections. We lumped these, along with plicate or a segmented margin, as descriptions of an umbrella-like pileus. The common current concept of turbinatus (turbinate) is a laterally obconical pileus. However, at that time turbinatus was also used to describe a spinning propeller-like disc of radiating wedges, like the vanes of a fan or windmill. When Linnaeus encountered AM-MIN (vide infra) he used the term infundibuliform to indicate its common laterally obconical cap. We believe it is significant that he did not use this term in any of his five descriptions of *A. umbellifer*, and therefore consider turbinatus as yet another way to describe a segmented umbrella-like cap. Size is not measured, but generally he uses minimus, and certainly parvus for species with a cap diameter around one cm or less. The stem is consistently described as long. Length is a relative term, in the case of agarics compared to cap diameter. Generally, a stem less than one cap diameter is considered short. A "normal" stem length varies from one to two cap diameters. Usually, the stem must approach or exceed three cap diameters, before "long" is used as

a reliable and unmistakable identifying character. Thus, his concept is an agaric with a stem noticeably longer than at least two and one-half cap diameters. In his formal treatments Linnaeus described the gills as broad-based, but did not describe them as decurrent. Neither cited illustration shows gill attachment; we interpret broad-based to have its usual meaning of attached adnate gills. Finally, the observation that the species grows among leaves seems significant to Linnaeus because he adds it separately on each of three occasions; such insistence should not be dismissed to indicate casual growth between random individual fallen leaves.

All cited authors lend strong support to the concept of a small white mushroom with a long stem. Two illustrations (Micheli 1729, reproduced here as Fig. 2A; Buxbaum 1733, reproduced here as Fig. 2B) indicate a mushroom with a stem considerably more than three cap diameters long. Neither has the flat cap (pileo plano, Linnaeus 1737; capitolo turbinato plano, Linnaeus 1741) described by Linnaeus on his two encounters with the species. Redhead & Kuyper (1987) commented on Micheli's description (pileo hemisphaerico), suggesting, "it appears certain that Linnaeus included more than one species in his concept", and "it may be presumed that Linnaeus included more than one
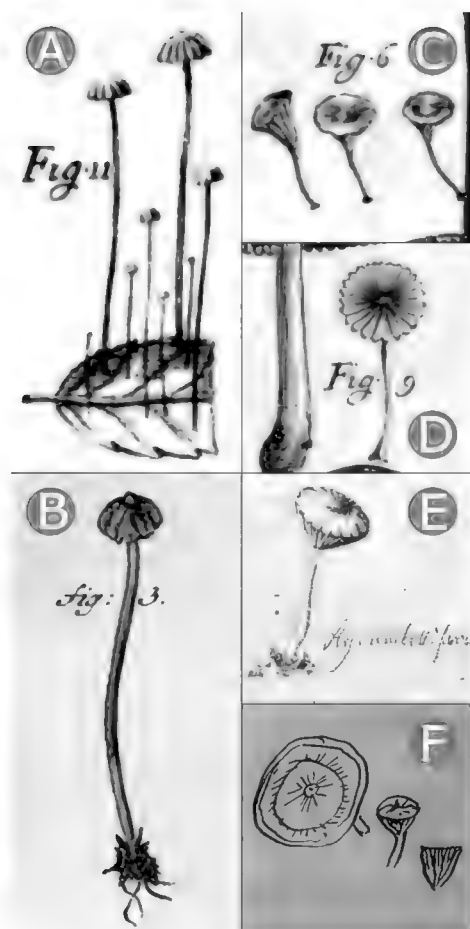
Fig. 2. Past images related or unrelated to *Agaricus umbellifer*: A. Illustration by Micheli (1729) cited by Linnaeus (1745, 1753, 1755); no doubt about the long stem and epiphyllic nature of the species; B. Illustration by Buxbaum (1733), cited by Linnaeus (1745, 1753, 1755), illustrating Linnaeus's concept of a long stem; C, D. Illustrations by Micheli (1729) available to but not cited by Linnaeus, of species much closer to AM-MIN than the image he selected; selecting the image shown in A, not the ones in C & D, shows that Linnaeus did not have an AM-MIN-like species in mind for his epithet *umbellifer*; E. Rudbeck's unlabelled illustration from his tour of Lapland (Anfält 1987), believed to represent AM-MIN; F. Linnaeus's sketch in his log of his tour of Lapland (Fries 1913), which he described with a phrase name, also believed to represent AM-MIN; note the similarity of E & F (and their similarity to C & D, but significant difference from A & B); clearly both Linnaeus and his mentor Rudbeck were familiar with AM-MIN; neither E nor F was cited in the description of *A. umbellifer*, again demonstrating that AM-MIN was not Linnaeus's species concept of *A. umbellifer*.

species in his concept as suggested by the synonymized descriptions." Because Linnaeus repeatedly described the cap as flat, it is unlikely that he selected these illustrations as good examples of conspecificity. Rather, we suspect (but cannot prove) that Linnaeus chose these to stress the concept of a long stem. Likely a second reason for choosing Micheli's illustration was because it clearly illustrates the epiphyllic nature of the species, the same reason Linnaeus cited Haller (1742), who described a small white mushroom arising **from** (out of) decomposing beech leaves (**ex** folio fagino putrido). Linnaeus's "inter folia" has been interpreted to mean among (in the sense of between) leaves rather than on them (Jørgensen & Ryman 1994), a point of view that does not fit comfortably with four observations. First, Jørgensen & Ryman themselves state that Linnaeus's attention to ecological detail was lax. Hence, the difference between "among", "on", and "from" may be part of this laxity, in which case assigning literal interpretation may lead to error. Secondly, it is clear that, lax or not, the association with leaves was significant to Linnaeus, because he repeated this character in all three formal treatments (Linnaeus 1745, 1753, 1755) giving it extra stress by inserting it separately at the end of each description, the only character that he felt important enough to add separately to the phrase names. Thirdly, in the protologue Linnaeus (1753) specified growth on **piled** decomposing leaves (folia **congesta**, semiputrida), a situation where growing on, rather than between leaves seems unavoidable. Finally, part of the reason Linnaeus selected certain works to cite must be because their descriptions or illustrations show features of the species he was trying to describe. That he cited Haller's description of an epiphyllic species twice and Micheli's similar illustration three times cannot be dismissed as random.

Linnaeus's experience adds two further characters to the species concept: somewhat uncommonly seen, yet sufficiently distinctive to be memorable. Linnaeus described only two encounters with the species he named *A. umbellifer* in nine years, once in Lapland and once in Öland; hence not overly common. Despite the nine-year hiatus between these encounters, he seemed to have recognized the species readily the second time; hence quite distinctive.

Testing the fit of Linnaeus's concept with Am-min

TABLE 2 compares Linnaeus's species concept of *A. umbellifer*, as developed above, with Am-min. They differ in shape, size, and colour, and have conflicting differences in stem length (relative to cap diameter) and substrate preference. While some of the differences in characters have been

the subject of past debate, this analysis leaves little doubt about their validity. The number of irreconcilable major characters between *A. umbellifer* and Am-min makes it clear that Linnaeus had a species other than Am-min in mind for *A. umbellifer*.

Table 2. Comparison of Linnaeus's species concept of *Agaricus umbellifer* and Am-min

	Basic color	Cap diameter	Cap shape, commonest	Stem length	Gill spacing	Substrate, preferred
Owingsia umbellifera	White	≤1 cm	Flat	≥3× cap diam.	distant/reduced	dead leaves
Am-min	Yellowish	>1 cm	Funnel	≤1.5× cap diam.	moderate	turf

The above is not a new discovery or an original conclusion. Redhead & Weresub (1978) documented it, and Redhead & Kuyper (1987), looking at the same information discussed here, came to the same conclusion. Further, they demonstrated that had Linnaeus wished to apply *A. umbellifer* to Am-min, he had images of species with an omphalinoid pileus and shorter stipe (two reproduced here as Figs 2C & 2D) available to him among Micheli's illustrations (Micheli 1729), that were far more Am-min-like than the one he chose (reproduced here as Fig. 2A).

Surely the most compelling evidence, not discussed earlier, that Linnaeus did not consider *A. umbellifer* to be Am-min, is that both Linnaeus and his mentor, Olof Rudbeck, **knew** Am-min. Both had undertaken Lapland journeys, where both had seen this iconic species of northern heaths, and both had made readily identifiable sketches of it in their respective logs. Rudbeck's 1695 log, Iter Lapponicum—skissboken från resan till Lappland, was published posthumously (Anfält 1987). There is no evidence that Rudbeck described or named the species, but he left an illustration (reproduced here as Fig. 2E). Linnaeus's log of 1732, also entitled Iter Lapponicum, was also published posthumously and translated into several languages; we refer to the second Swedish translation by T.M. Fries (1913). In his log Linnaeus left an illustration (reproduced here as Fig. 2F) with a descriptive phrase name, *Amanita minima, infundibuliformi plana, lamellis alternis integris, bifurcates, alternis semi, alba*, the source (as mentioned) for our contraction Am-min. Note that Linnaeus applied *Amanita* quite differently from its current usage. The name was introduced by Dillenius (1719) for one of four genera of fungi, the genus with pileus, stipe, and lamellae—in other words the group we now refer to as agarics. Linnaeus admired Dillenius, spent a month with him in

Oxford, and often followed his classification. As we have seen, Linnaeus had no difficulty citing his own observations, or those of others, so that omitting both his mentor's and his own observations of Am-min should end any speculation that he intended to apply *A. umbellifer* to Am-min. All Linnaeus's descriptions and citations were intentional, carefully chosen to circumscribe the characters of his species concept accurately and precisely; the evidence that they should not be dismissed as random events due to oversight seems overwhelming. The conflict of Am-min with the original description of *A. umbellifer* leads us to conclude that both earlier lectotypifications of this name (vide infra) should be rejected according to Art. 9.19(c) of the Code.

Why then, after rather clear demonstration by Redhead & Weresub (1978) and Redhead & Kuyper (1987) that applying Linnaeus's epithet to Am-min was incompatible with the protologue, did this epithet still get applied to Am-min? Two years after Redhead & Kuyper's opinion, where they typified *A. umbellifer* with the illustration by Micheli cited by Linnaeus, Jørgensen & Ryman (1989) proposed Rudbeck's aquarelle of Am-min (reproduced here as Fig. 2E), as lectotype for *A. umbellifer*, stating, "Judging from the **descriptions** and the **circumstances** (cf. Linnaeus 1737: 352, point 1) it is likely that Linnaeus based the description entirely on this drawing." Coming to this debate over 30 years later, without prejudice for either side (until we began this enquiry, we had accepted *Lichenomphalia umbellifera* as the appropriate name for Am-min, and had not been aware of this controversy), we developed Linnaeus's species concept from his writings (Tab. 1), and compared that with Am-min (Tab. 2), which led us to conclude that Linnaeus's description of *A. umbellifer* does not fit Am-min. Consequently, we cannot agree with Jørgensen & Ryman (1989) that Linnaeus's **descriptions** suggest this drawing was used to create the protologue. The **circumstances** Jørgensen & Ryman (1989) refer to, citing Linnaeus's introduction to the section dealing with *Fungi* in his Flora Lapponica, consist of references by Linnaeus to a fire that destroyed many books and good source material, and grateful expressions of relief that many of Rudbeck's illustrations had been kept safe from fire. Linnaeus thanks Rudbeck for putting these ("eos", note the plural) at his disposal. Thus, certainly Linnaeus acknowledges the availability of Rudbeck's material for his book, but speaks of illustrations in the plural (as do Jørgensen & Ryman), and nowhere does Linnaeus mention that any particular illustration was used for the description of any particular species. There is no record from Linnaeus or others that Rudbeck's unnamed and unpublished drawing of an undescribed

species—or any particular illustration—was the basis for species #471 in his Flora Lapponica, the protologue, or elsewhere. There is a vast gap between having **several** illustrations at one's disposal, and using one specific illustration to describe one specific species. Nothing in Jørgensen & Ryman's subsequent two paragraphs support their changing the probable "it is likely" in the above quote to the certain, "we have also **proved**" that Linnaeus had Am-min in mind when describing *A. umbellifer*—in fact, quite the opposite—and we find nothing to support referring to Rudbeck's drawing as "original material" for *A. umbellifer*.

We do not claim that Linnaeus did not have access to Rudbeck's Lapland material, including the drawing under question. The logical assumption would be entirely opposite. Olof Rudbeck fil. (1660–1740) was a Swedish scientist and explorer, who joined an expedition to Lapland commissioned by the King of Sweden. At that time Lapland still had the draw of terra incognita, even in Scandinavia. Unfortunately, most of Rudbeck's collections and many records from that journey were lost in a fire, but some paintings and his journal-sketchbook survived to be published posthumously. Rudbeck was mentor to the young Linnaeus, when the latter began his pursuit of botany and medicine. It is easy to imagine that the exciting tales of the mentor, supplemented by notes and illustrations, kindled enthusiasm for Lapland in his young student. Our guess is that Rudbeck made the material available to Linnaeus already before the latter's own Lapland journey, and possibly these helped spur the 25-year-old Linnaeus to undertake a Lapland journey of his own. It is equally reasonable to assume that his mentor continued to share his tales, notes, and records of the earlier journey with his protégé after the latter's return, earning him the thanks noted by Jørgensen & Ryman. Also, there is no reason to doubt that the unpublished material that Rudbeck put at Linnaeus's disposal contained the lovely illustration of Am-min. The description of species #471, however, especially of its long stem and its substrate, does not fit Rudbeck's illustration (stem measured just under 1.5× cap diameter, substrate seeming to be mossy soil), making it extremely difficult to accept that one was based on the other.

While we find no similarity between Linnaeus's description of *A. umbellifer* and Rudbeck's illustration, we note the uncanny similarity of Rudbeck's illustration (reproduced here as Fig. 2E) to Linnaeus's sketch of Am-min (reproduced here as Fig. 2F) in the log of his own Lapland journey. Linnaeus's notes described Am-min as infundibuliform, which fits both his and Rudbeck's

sketches, but neither he nor authors he cited ever used that term to describe *A. umbellifer.* Linnaeus made no suggestion then or later that this sketch represents his concept of *A. umbellifer.* The two logbooks offer clear proof that both Rudbeck and Linnaeus knew Am-min, but that it was not the species to which Linnaeus applied the binomial *Agaricus umbellifer.* Such suggestions were made later by others. In the case of Rudbeck's illustration, somebody other than Rudbeck wrote, "*A. umbelliferus* L. Fl. Su. v II 1192" on the illustration, referring to *Agaricus umbellifer,* species #1192, p. 440, in Linnaeus's Flora Suecica vol. 2 (Linnaeus 1755). Jørgensen & Ryman (1989) quoted Nilsson's opinion (Nilsson 1987) that this was added by the botanist Carl Johan Hartman, either 1811–1814 or 1841. Our guess is that this was not a novel idea by Hartman, but that he was influenced in this by a common misunderstanding that Fries (1821) synonymized *Agaricus ericetorum* Pers. with *A. umbellifer* L. (vide infra). If that is so, and if those are the only possible dates of the annotation, then the likely year was 1841. In the case of Linnaeus's sketch in his Iter Lapponicum, the species is identified as "*Agaricus (Omphalia) umbellifer* Fr." not by Linnaeus, but by T.M. Fries in his 1913 Swedish translation. Parenthetically, it is worth noting that there is no valid taxon, "*A. umbellifer* Fr." Fries treated the species twice (Fries 1825, 1828), and both times made it clear that he was referring to the taxon of Linnaeus.

Both Redhead and Kuyper remained silent, effectively ending the debate. It took over a decade before Redhead, with different collaborators (Redhead & al. 2002), published a major revision of omphalinoid genera, which required, inter alia, nomenclaturally suitable type species to be assigned to *Arrhenia* and *Omphalina,* while circumscribing Am-min as the type species for their newly erected genus, *Lichenomphalia,* created for lichenized omphalinoid basidiomycetes. This was accomplished by synonymizing and otherwise resolving various competing names and interpretations for *A. umbellifer* and *A. ericetorum.* To do this, they took advantage of a recent change to the International Code of Botanical Nomenclature (Greuter & al. 2000), which now permitted acceptance of the lectotype declared by Jørgensen & Ryman, namely Rudbeck's illustration. Redhead & al. rejected Redhead & Kuyper's earlier typification of *A. umbellifer,* and accepted Jørgensen & Ryman's instead, thereby applying the epithet coined by Linnaeus to Am-min. This contrivance avoided debate in solving the systematics of the genera involved—including getting smooth acceptance of their new genus *Lichenomphalia*—at the expense of ignoring

interspecific characters of morphological diversity. Neither Redhead & Kuyper, jointly or separately, nor Redhead and his new collaborators, ever mentioned, challenged, rebutted, refuted, or withdrew Redhead & Kuyper's published observations regarding the painfully catachrestic application of *umbellifer* to Am-min. Further, Redhead & al. (2002) did not embrace Jørgensen & Ryman's claim that the Rudbeck sketch was the only image on which Linnaeus based his description of *A. umbellifer*. Rather, they noted the sketch had been "APPARENTLY available to Linnaeus." This statement certainly did not support the claim that the illustration was part of the original material, and Redhead & al. (2002) offered no discussion whether such availability equated to being even a partial source, let alone the **sole** source for Linnaeus's conflicting description. Further, they did not claim the epithet fit the description in any way, but rather referred to it as a "**compromise**", one they hoped would "resolve and end a 250-year old controversy over these names." Thus, the problems created by accepting *A. umbellifer* as the basionym of Am-min remained exactly as Redhead & Kuyper (1987) had outlined them earlier (and we confirmed here): the epithet remained misapplied to Am-min.

In summary, *Agaricus umbellifer* was lectotypified by Jørgensen & Ryman (1989) in the belief that the designated lectotype, Rudbeck's unnamed and unpublished (in his lifetime) 1695 illustration, was part of the original material on which Linnaeus based his protologue description. After careful study, we have found no evidence to substantiate this belief. All available evidence supports the diametric view, namely that Linnaeus did not base his description on this illustration, and had a considerably different species in mind for the name. That this must be so is confirmed by the major conflicts produced from the (mis) application of *A. umbellifer* to its declared lectotype. Subsequent acceptance of that lectotypification by Redhead & al. (2002) did not alter its legitimacy, because all available evidence still suggested that Rudbeck's illustration was not the original material for the protologue of *A. umbellifer*, and Linnaeus did not use it as a basis for his protologue—a matter, as pointed out, that Redhead & al. (2002) were careful to skirt. We reject this lectotype by Jørgensen & Ryman because there is no evidence it was used as original material by Linnaeus, and it is in major conflict with Linnaeus's protologue description. This rejection automatically rejects the subsequent adoption of Rudbeck's illustration as lectotype by Redhead & al.

One earlier lectotype remains to be considered. In his Systema Mycologicum, Fries (1821: 160) had referred Micheli's illustration, cited by Linnaeus, to *A. capillaris* Schum. [≡ *Mycena capillaris* (Schum.) P. Kumm.]. Both drawing and description seem to fit that species. Redhead & Kuyper (1987) proposed Micheli's illustration as lectotype for *A. umbellifer*. The illustration shows a hemispheric cap and the description states "capitolo haemispherico"—the very character that caused Redhead & Kuyper to wonder whether Linnaeus embraced more than one species in his concept! We reject this lectotypification because it contradicts the protologue description, a contradiction emphasized but not resolved by the proposing authors.

Search for a species to fit Linnaeus's concept of *Agaricus umbellifer*

Background

On a trip to Lapland in 2006, the senior author collected a small white epiphyllic agaric with a membranaceous, translucent, flat, umbrella-like segmented cap, and distant gills, supported by a long white stem, arising from that year's fallen leaves of *Populus tremula* L. (Fig. 3). He identified the collection tentatively as *Marasmius tremulae* Velen. and placed it in his personal herbarium. There it remained as collection 06.10.04.av01, altogether forgotten until this study gave rise to a lucid picture of Linnaeus's species concept of *A. umbellifer*. Immediately, this collection came to mind. On review, the collection shared all the criteria of Linnaeus's *A. umbellifer* (Tab. 1, bottom row), including the shape of the cap, which had been a problem in the past. To learn whether this species, fitting Linnaeus's description, could be the species Linnaeus described, we undertook to determine whether the species is prevalent in the regions explored by Linnaeus; in other words, is it likely that Linnaeus would have encountered this same species?
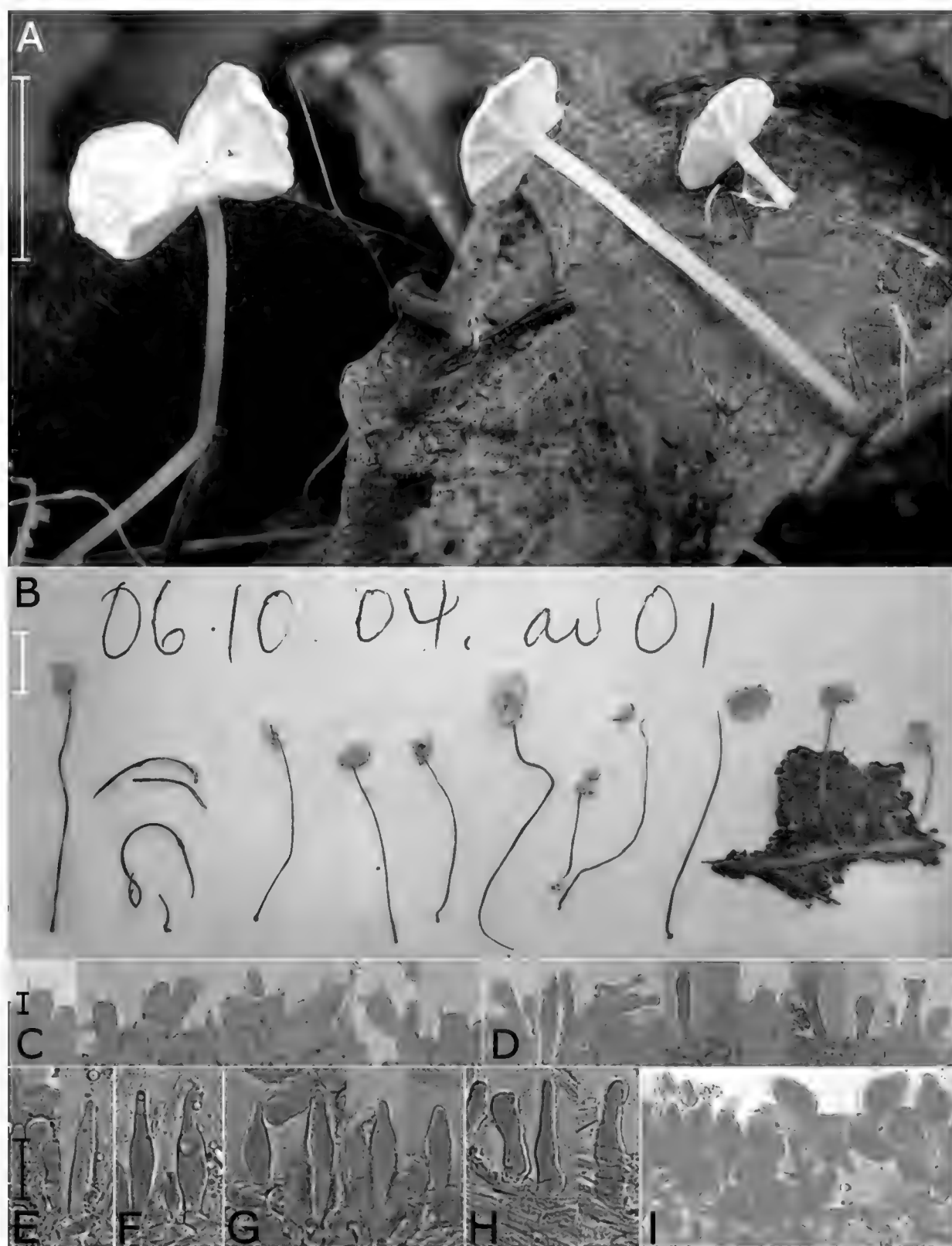
FIG. 3. *Owingsia umbellifera* (A–D = neotype, O-F-76596; E–I = TUF118289): A: Basidiomata on fallen leaves of *Populus tremula*. Note the white, flat, pellucid, umbrella-like caps, long stem, equal to about three cap diameters, widely spaced gills, broader at their base, and the many white rhizomorphs/sterile stipes. B: Neotype collection exsiccatum. C: Basidia, mostly four-spored, with about 15% 2-spored (not due to focal length artefact). D: Cystidia. E: Cheilocystidia. F: Pleurocystidia. G: Caulocystidia. H: Pileocystidia. I: Pileipellis elements. Scale bars: A, B = 5 mm; C, D = 5 μm; E–I = 25 μm.

Method

Collections identified as *M. tremulae* or *M. epiphyllus* (Pers.) Fr. from Lapland, Öland, and surrounding areas, augmented by a few from other regions (Tab. 3), were sought for molecular studies. Related sequences deposited in GenBank and UNITE (Kõljalg & al. 2013, Nilsson & al. 2019) were added to the analysis to construct the phylogeny (Fig. 4). ITS-DNA processing followed Voitk & al. (2020) and phylogenetic analysis Voitk & al. (2022). New sequences were deposited in UNITE and/or GenBank. Specimens were vouchered in the Herbarium, University of Oslo, Norway (O), the Fungarium, University of Tartu, Estonia (TUF), and the Herbarium, University of Western Ontario, London, Canada (UWO).

TABLE 3. Collections and sequences used in phylogenetic analyses. Neotype in bold print.

SPECIES	Country	FUNGARIUM NO. (DUPLICATE NO.)	ITS	PUBLICATION
Owingsia umbellifera	Czech Rep.	BRNM695733	FN293008	Antonín & al. 2010
	Czech Rep.	PRM902346	FN293010	Antonín & al. 2010
	Czech Rep.	PRM894159	FN293012	Antonín & al. 2010
	Estonia	TUF106979	UDB015277	UNITE
	Estonia	TUF118289	UDB015455	UNITE
	Estonia	TUF118453	UDB018190	UNITE
	Finland	OULU-F-16597	UDB07672480	**This study**
	Finland	OULU-F-16603	UDB07672482	**This study**
	Finland	OULU-F-16606	UDB07672483	**This study**
	Finland	OULU-F-24076	UDB07672485	**This study**
	Greenland	NN048205a	JN943599	Schoch & al. 2012
	Italy	BRNM695779	FN293007	Antonín & al. 2010
	Norway	OULU-F-21543	UDB07672484	**This study**
	Norway	**O-F-76596 TUF117828**	**UDB0799033**	**This study**
	Norway	O-F-21830	UDB036647 NOBAS2883-16	UNITE
	Poland	FeF427	MZ493079	Bilanski & Kowalski, unpubl.
	Russia	NN050222a	JN943602	Schoch & al. 2012
	Slovakia	PRM870457	FN293009	Antonín & al. 2010
	Slovakia	BRNM523367	FN293011	Antonín & al. 2010
	Sweden	UPS-F-740369	UDB07672488	**This study**

Species	Country	Fungarium no. (duplicate no.)	ITS	Publication
	UK	K(M)40466	FN293013	Antonín & al. 2010
	USA	MF80535 NS3148	OM906872	Mohatt & al., direct sub.
Owingsia sp. 1	Estonia	TUF118324	UDB015484	UNITE
	Estonia	TUF118729	UDB019538	UNITE
	Finland	OULU10007053	UDB07672481	**This study**
	Finland	OULU-F-24077	UDB07672486	**This study**
	Slovakia	BRNM523372	FN293016	Antonín & al. 2010
Owingsia sp. 2	Canada	UWO-F3413	OP290397	**This study**
Owingsia sp. 3	Czech Rep.	BRNM695419	FN293017	Antonín & al. 2010
	Japan	soil sequence	MT594707	Favero Longo & al., unpub.
Other *Physalacriaceae*				
Armillaria mellea	—	PBM2470 AFTOL-449	AY789081	Binder & al. 2006
Dactylosporina steffenii	Costa Rica	TENN58785	HM005073	Petersen & Hughes 2010
Desarmillaria tabescens	USA	00i-99	AY213590	Kim & al. 2006
Flammulina velutipes	—	7200	AF030877	Hughes & al., unpub.
Gloiocephala epiphylla	USA	DED5971	DQ097357	Binder & al. 2006
Hymenopellis radicata	Sweden	TENN62837	GQ913377	Petersen & Hughes 2010
Laccariopsis mediterranea	Italy	MCVE23445	JX271808	Vizzini & al. 2012
Mucidula mucida	Austria	TENN59324	GQ844235	Petersen & Hughes 2010
Paraxerula americana	USA	DBG21746	HM005143	Petersen & Hughes 2010
Rhizomarasmius pyrrhocephalus	USA	TENN51091	DQ097369	Binder & al. 2006
Rhodotus palmatus	Czech Rep.	PRM889504	KC179739	Tang & al. 2014
Strobilurus conigenoides	USA	TENN61318	GQ892821	Petersen & Hughes 2010
Xerula pudens	Estonia	TUF117431	UDB031394	UNITE
Outgroup				
Crinipellis scabella	—	CBS243.53	MH857177	Vu & al. 2019
Marasmius rotula	Denmark	NN005958	JN943598	Schoh & al. 2012

[a] Personal herbarium of Sara Landvik
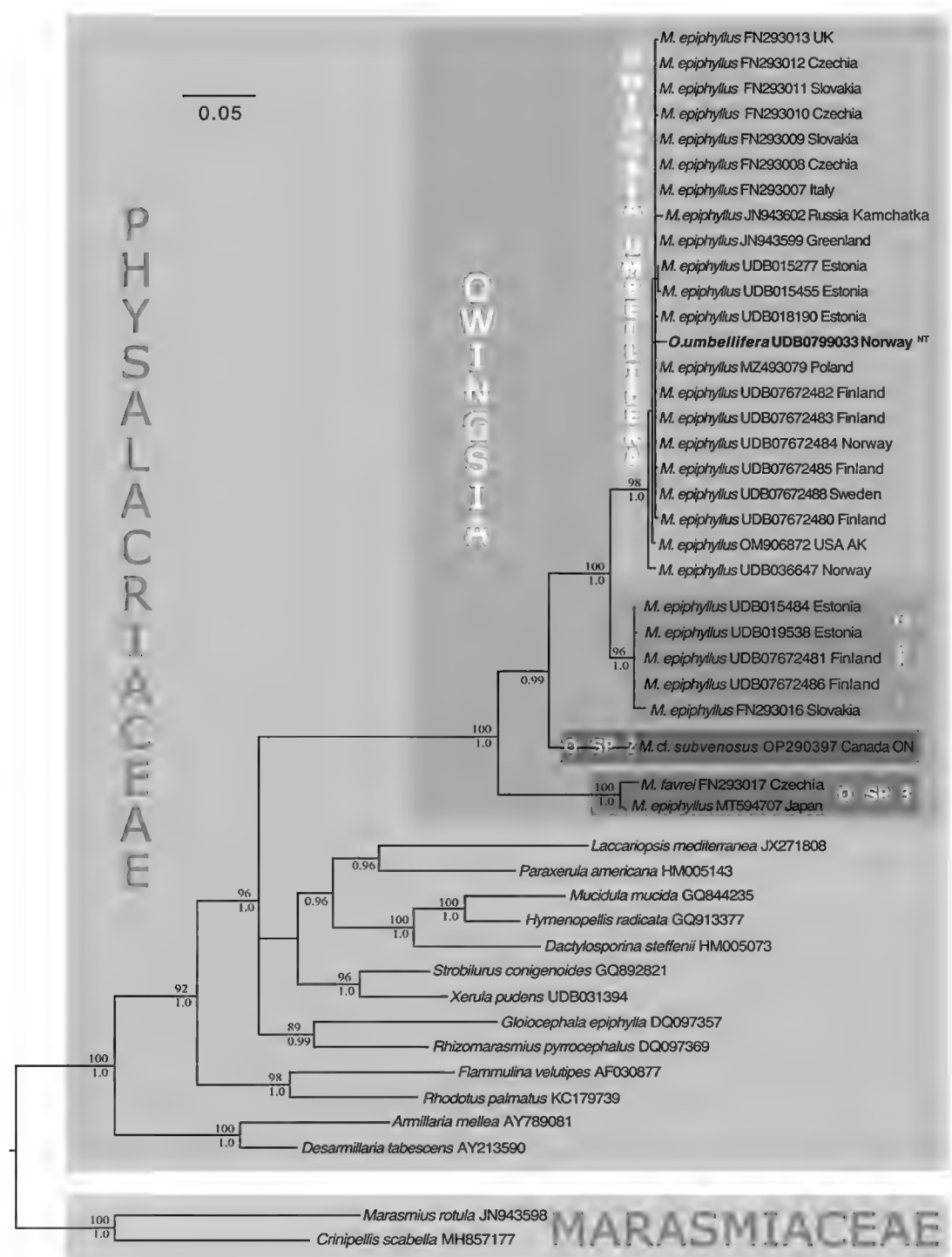
FIG. 4. ITS-based phylogenetic pathways, showing the placement of *Owingsia* in *Physalacriaceae*, here sister to *Marasmiaceae*, containing *Marasmius* s.str. Bayesian analysis shows that the genus contains *O. umbellifera* and three other clades of unidentified species to which the name *M. epiphyllus* has been applied. ML bootstrap support ≥70% and the Bayesian posterior probabilities ≥95% are shown above and below the branches (bs/pp), respectively. Maximal likelihood analysis revealed *Owingsia* spp. 2 and 3 in a single clade with 84% support. Sequences are identified by the name recorded on the genetic depository or fungarium accession database, with the exception of the neotype, identified by its current name. This limited sampling is inadequate to resolve the genus, a question beyond the scope of this work. *Owingsia umbellifera* is widely distributed, documented in North America from Alaska and Greenland, and in Eurasia from Scandinavia to Kamchatka. The neotype from the Norwegian part of Lapland appears in bold print. The two sister species, *O. umbellifera* and *Owingsia* spp 1, were sympatric, and several recorded on the same substrate, dead leaves of *Populus tremula*.
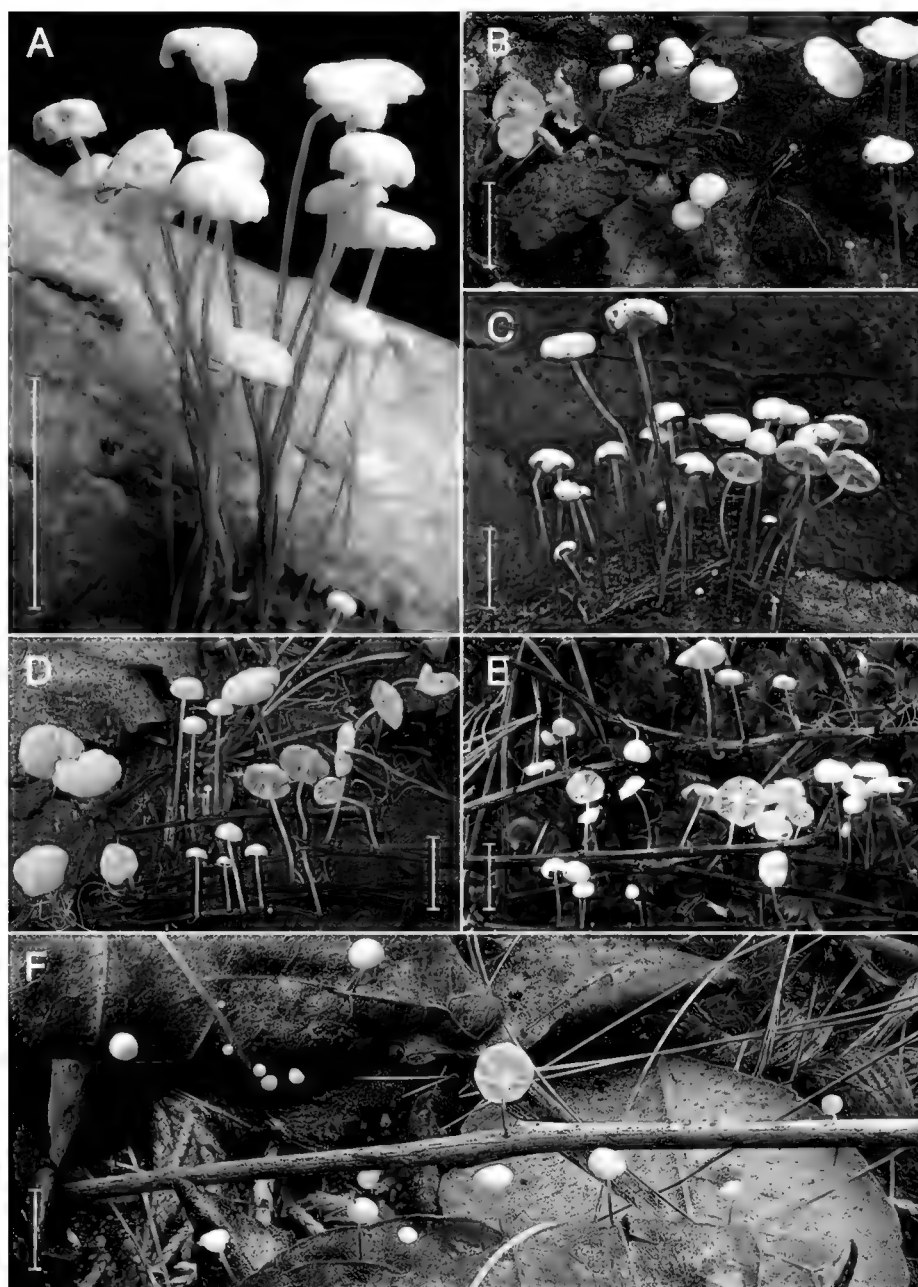
Fig. 5. *Owingsia* species from three of our four species clades. ***Owingsia umbellifera***, Estonia: A. TUF118453; B. TUF118289; C. TUF106979. ***Owingsia* sp. 1**, Estonia: D. TUF118729; E. TUF118324. ***Owingsia* sp. 2**, Canada: F. UWO-F3413. Scale bars = 10 mm. [Photos A–E courtesy of Vello Liiv.]

Results

Bayesian analysis (Ronquist & al. 2012) showed that *Marasmiaceae*, containing the genus *Marasmius*, formed a sister clade to *Physalacriaceae*, where our specimen fell, with 29 other sequenced specimens, which split among four species (Figs 4, 5). No other collections were identified as *M. tremulae* and 27 of the 30 were identified as *M. epiphyllus*. Maximum likelihood analysis (Stamatakis 2014) of the same material placed species 2 and species 3 into a single clade with 84% support. The genus where these sequences clustered is undescribed, but sequences from other studies indicated that this clade has been identified as *Marasmius* sect. *Epiphylli* Kühner in various studies (Owings 1997, Owings &

Desjardin 1997, Wilson & Desjardin 2005, Jenkinson & al. 2014). Our collection from the Norwegian part of Lapland fell into the largest species clade with 21 other specimens, distributed widely throughout the Northern Hemisphere: from Alaska to Greenland in North America, and from Scandinavia to Kamchatka in Eurasia, with additional collections from central Europe. Figure 6 plots collections of this species from both regions where Linnaeus recorded it, Lapland and the Baltic Sea. Unfortunately, two relatively recent collections from Öland failed to yield amplifiable DNA, but collections from nearby Saaremaa and the west coast of Estonia produced sequences that fell into the same clade. The species is sister to a small clade of five collections, with which it shares morphology (as evidenced by the application of the same name), distribution in Lapland, and substrate preference (fallen leaves of *Populus tremula*).

Fig. 6. Map of Fennoscandia and environs, showing the origin of sequenced specimens of *Owingsia umbellifera* (yellow circles; star for neotype) from the two sites where Linnaeus collected them, Lapland (green hatching) and Öland (Ö). Specimens from Öland did not yield DNA, but specimens from nearby Saaremaa (S), and the west coast of continental Estonia, both in the Baltic Sea, are shown instead.

Comments

Our investigations confirm and add further supporting evidence to past conclusions that the epithet *umbellifera* is misapplied to its current taxon. We were unable to substantiate previous claims to the contrary. We identified a species that matched Linnaeus's concept without conflict, and confirmed its prevalence in the same regions where Linnaeus collected his *A. umbellifer*. In our brief survey of likely candidates, this species is the most common among several similar species we now know to exist in the group, and a review of FIG. 5 suggests this species has the longest stipe of the group (i.e., most closely resembles the illustrations chosen by Linnaeus to show this character of his species). These two observations make it most likely that of the group of similar species, this was probably the one seen and described by Linnaeus with the name *A. umbellifer*. These observations do not require further comments. However, how to handle them in a work devoted to the nomenclature of two epithets warrants contemplation.

Initially we recombined *A. umbellifer* into *Marasmius*, but discussion during the review process convinced us that this was less than optimal, given the phylogenetic distance of *Marasmius* s. str. from this genus. In an unpublished Master's thesis, Owings (1997), using the LSU marker, first showed that *Marasmius*, as known at that time, was a polyphyletic genus, and, inter alia, that species of *Marasmius* sect. *Epiphylli*, along with some other genera, followed a divergent evolutionary pathway to the *Physalacriaceae*. She reported these findings with her supervisor in an abstract (Owings & Desjardin 1997), and her observations have been confirmed in various LSU-based studies of the *Physalacriaceae* (e.g., Wilson & Desjardin 2005, Ronikier & Ronikier 2011, Vizzini & al. 2012, Jenkinson & al. 2014). Multilocus analysis by Matheny & al. (2006) confirmed that *Marasmiaceae* and *Physalacriaceae* form separate provisional families arising in what they named the marasmioid clade, one of six major clades of *Agaricales*. Classifying *Marasmius*, Kühner (1933) named "*M.* sect. *Epiphylleae*", forming the name from *M. epiphyllus*, which he placed in the section. The Code (Art. 10.8) considers such implicit assignment of type species valid; Kühner's sectional epithet must be corrected to a masculine plural adjective, agreeing with the masculine genus (Art. 21.2) — *Marasmius* sect. *Epiphylli*.

A new genus typified by *Agaricus*/*Marasmius epiphyllus* would require a satisfactory typification of the species. A beginning was made by Singer (1949), who described *M. epiphyllus* as heterogenous, discussed two taxa, and then stated that he had no hesitation to designate one collection from Ulfült near

Femsjö as topotype for *M. epiphyllus*. This is a bit puzzling, because topotypes have no nomenclatural significance, and are not **designated**, but are what they are by definition: the same species from the same site where the type was collected. In this case, neither Persoon, who described *Agaricus epiphyllus*, nor Fries, who sanctioned that name and subsequently transferred it to *Marasmius*, cited a type collection, making it impossible to meet the definition. Fries did indicate that he had seen the species (v. v.), but the Code requires that a precise specimen be identified by the author for valid typification (Art. 7.11). As opposed to topotypes, neotypes must be designated. Singer did not **designate** the cited specimen as neotype, but rather stated, "we … **recommend** it as neotype of the species." Characteristically, Singer's type designations are brief, clear, and unequivocal, supporting the contention that such was not his intent for *M. epiphyllus*.

Erecting a new genus with a new name and its own type species circumvents the above concerns nicely, and also avoids the need to reconcile some additional concerns. For example, Desjardin (1989) described lack of rhizomorphs as one of the characters of *M.* sect. *Epiphylli*, but rhizomorphs are very prominent in Fig. 3. Needless to say, the simplicity of erecting a new genus appealed to us. We shall describe briefly the new genus, *Owingsia*, recognizing that a genus created for nomenclatural convenience, without resolving its taxonomy, will not satisfy all taxonomic expectations until its taxonomy becomes settled. Taxonomic study to resolve *Marasmius* sect. *Epiphylli* is a major undertaking, requiring familiarity with the group, wide sampling and sequencing, reconciling several old names by typification, synonymization, or other means, and probably needs a global approach. For example, the different topology we noted with Bayesian and maximum likelihood analysis suggest the need for including more North American specimens, ideally with an attempt to sequence the type of *Marasmius subvenosus* Peck, and the need to include more (conservative) genetic markers in the analysis. Such work, best done by experts familiar with these species, is well beyond the scope of the stated narrow aims of this nomenclatural study. Our only reason to erect it at this time is to accommodate the new combination for *A. umbellifer* in a place more logical than *Marasmius*.

The final question warranting some comment is whether *Agaricus epiphyllus* could be a later synonym of *A. umbellifer*. Should these two sanctioned names prove to be synonymous, the normal rules of priority would apply (Art. 11.4; Art. F.3), and *A. umbellifer* would be the correct name. Pre-DNA work lacked the advantages available to us. For example, specimens FN293007–FN293013 in our phylogenetic tree are conspecific with our newly designated neotype and

were used by Anton*ín & Noordeloos (1993*: 52–57) to describe *M. epiphyllus*. However, their specimens examined also contain FN293017 that fell into the Sp. 3 clade in our tree, and a much larger number of specimens that have not been placed phylogenetically. Earlier concepts require revision, in light of new technology, before this question can be answered. What we can say at this time, is that because our limited sequencing produced three clades to which the epithet has been applied, likely the taxonomist(s) who undertake(s) this task should have some latitude with applying the name to retain it without conflict, if desired.

Taxonomy

> *Owingsia* I. Saar, Voitk & Thorn, **gen. nov.**
> MB 845593
>
>> = *Marasmius* sect. *Epiphylli* Kühner [as "*Epiphylleae*")], Le Botaniste 25: 93. 1933.
>>
>> Differs from *Marasmius* s.str. by its acollariate attached lamellae, stipe with visible vestiture, lack of broom cells, prominent cystidia, and molecular data.
>>
>> TYPE SPECIES: *Owingsia umbellifera* (L.) Voitk & al.
>>
>> ETYMOLOGY: Named after Pamela Owings, who first described the divergence of *Marasmius* sect. *Epiphylli* from the evolutionary pathway of the core *Marasmius* group.

Basiomata of the four putative species clades that nestled in *Owingsia* by our ITS data (FIG. 5) are all small (cap diameter seldom over 10 mm) and whitish, with all tissues inamyloid, non-dextrinoid, and share the following characters: pileus segmented, somewhat parasol- or windmill-shaped, most commonly plane at maturity; lamellae reduced to widely spaced, acollariate, developing fold- or vein-like anastomosing ridges, developed lamellae approach the stem for a broad attachment, but within a fraction of a mm develop a sharp notch, attached to the upper stipe less broadly; stipe usually long (>2.5× cap diameter), central, pruinose, insititious; basidiospores fusiform, hyaline, without iodine reactions; cystidia present on lamellar sides and edges, on stipe, and on pileus, narrowly fusiform to narrowly lageniform, mostly with a long neck, thin-walled; pileipellis hymeniform, made up of clavate or broadly clavate, slightly to distinctly thick-walled cells; stipitipellis a cutis; epiphyllic on fallen deciduous leaves and small deciduous or herbaceous litter. Phylogenetically, our ITS data (FIG. 4) show that the genus arises from a well-supported pathway within the *Physalacriaceae* (distant from *Marasmius*, type species *M. rotula*).

> SPECIMENS EXAMINED: For specimens of *O. umbellifera*, see below.
>
> SP1: **ESTONIA**, SAARE, **Saaremaa**, Harilaid, 02.11.2011, Vello Liiv (TUF118324; UNITE

UDB015484); near Viidu, mixed forest, on leaves of *Populus tremula*, 08.10.2013, Vello Liiv (TUF118729; UNITE UDB019538); **FINLAND**, Outer Ostrobothnia, Ylitornio, S end of Kuusikkorommas, S part of the nature protection area, rich spruce-dominated mixed forest on calcareous ground, on leaves of *Populus tremula*, 27.09.2014, Esteri Ohenoja, Taina Romppanen, Lasse & Marja Tuominen (OULU10007053; UNITE UDB07672481); Sompion Lappi, Pelkosenniemi, Jaurujoki E, Kuotelonjoki SW, 26.08.1994, Ulla Nummela-Salo, Pertti Salo (OULU-F-24077; UNITE UDB07672486).

SP2: **CANADA,** Ontario, **Essex County**, Point Pelee National Park, West Beach, 41.9266 -82.5138, 176 m a.s.l., on fallen leaflets and rachis of *Ptelea trifoliata* in open grass-oak-juniper savannah on shoreline, 05.10.2020, P. Kelly, N.M. Weerasuriya & R.G. Thorn, RGT201005/08 (UWO-F3413; OP290397; culture DAOMC252643).

Comment. Formation of a distinct genetic clade within *Physalacriaceae* and congruence with *Marasmius* sect. *Epiphylli* has been confirmed by previous work (vide supra). A fuller and more exact concept of the genus awaits further taxonomic work within the group, including typification of its species.

Owingsia umbellifera (L.) Voitk, I. Saar & Thorn, **comb. nov.**   Fig. 3
MB 845594

≡ *Agaricus umbellifer* L. [as "*umbelliferus*"], Sp. Pl. 2: 1175. 1753 (nom. sanct., Fr., Elench. Fung. 1: 22. 1828).

Type: **Holotype**, none. **Neotype** [**here designated**, MBT 10009104], Norway (Lapland), Finnmark (now Troms og Finnmark), Rafsboten, Tverrelven, 70.0159°N 23.5587°E, 47 m asl, in mixed woods on fallen leaves of *Populus tremula*, leg. Andrus Voitk 06.10.04. av01 (O-F-76596; **isoneotype** TUF117828; UNITE UDB0799033).

Macroscopic. Basidioma: small, white, epiphyllic, with a thin, translucent, flat, umbrella-like pileus on a long stipe; pileus: 4–8 mm in diameter, membranaceous, translucent, plane to gently dome-shaped, becoming plane with maturity (Figs 3A, 5B), drooping during drying (Fig. 3C, 5A, C), but on rehydration flattening out again, umbrella-like segmented, white; lamellae: distant, occasionally reduced, develop cross-veining anastomoses with age, approaching the stem for a broad attachment, but form a deep notch a fraction of a mm away from the stem (possibly by separating from it) to attach more narrowly to the upper stipe, white, acollariate; stipe: 15–26 mm high and about 1 mm wide, evenly cylindrical, straight or occasionally bent, minutely flocculose, white, with some yellow to straw colour rising from the base with maturity, insititious, associated with several white rhizomorphs or sterile stipes (Fig. 3A, B); spore print: white.

Microscopic. Basidiospores: (n = 50; 3 basidiomata, 2 collections, 2 observers) 7.7–13.5 × 3.5–6.7 µm (average 11.8 × 5.1 µm), Q = 2.0–2.9, (average 2.3),

ellipsoid-lacrymoid, hyaline, inamyloid; basidia: (n = 7) 8–11 × 42–54 μm, 4-spored, about 15% 2-spored (Fig. 3C); cystidia: plentiful and pleomorphic (Fig. 3D); cheilo- and pleurocystidia: similar (n = 18) 25–42 × 4.5–67.3 μm (average 33.5 × 5.7 μm), narrowly fusiform to lageniform, mostly with a long neck, thin-walled (Fig. 3E, F); pileocystidia: slightly larger but otherwise similar to hymenial cystidia (n = 12) 27–44 × 5.3–8.5 μm (average 38 × 6.6 μm), narrowly fusiform to narrowly lageniform, mostly with a long neck, thin-walled (Fig. 3G); caulocystidia: (n = 8) 20–26 × 4–6 μm (average 24 × 5 μm), fusiform to lageniform, thin-walled to slightly thick-walled (Fig. 3H); pileipellis: hymeniform, (n = 28) 12–26 × 6–12 μm (average 19 × 10 μm), clavate to broadly clavate, slightly to distinctly thick-walled cells (Fig. 3I); stipitipellis: a cutis of hyphae, up to 3–8 μm wide; clamp connections: in all tissues.

> ADDITIONAL SPECIMENS EXAMINED: **ESTONIA**, SAARE, **Saaremaa**, near Viidu, on rotten wood, 10.10.2010, Vello Liiv (TUF106979; UNITE UDB015277); on deciduous twigs, 28.09.2012, Vello Liiv (TUF118453; UNITE UDB018190); PÄRNU, **Pärnumaa**, Nigula mire, swamp forest, on fallen leaves of *Populus tremula*, 03.10.2011, Vello Liiv (TUF118289; UNITE UDB015455). **FINLAND**, INARIN LAPPI, Utsjoki, Kenesjärvi, 14.09.1972, Martti Ohenoja (OULU-F-16606; UNITE UDB07672483); PERÄ-POHJANMAA, **Lapland**, Tornio, Kalkkimaa SE, near Alatolo farm, S side of the road, deciduous forest (*Alnus*), 16.09.1986, Esteri Ohenoja, Tuula Vuorinen (OULU-F-16597; UNITE UDB07672480); SOMPION LAPPI, Pelkosenniemi, Suvanto NW, Niskakorpi, Niskaojan varsi, N-side of road, 11.09.1985, Esteri Ohenoja (OULU-F-16603; UNITE UDB07672482); Pelkosenniemi, Siulioaapa NE, 25.08.1994, Ulla Nummela-Salo, Pertti Salo (OULU-F-24076; UNITE UDB07672485). **NORWAY**, TROMS, Lullesletta, rich deciduous forest along a brook, 19.08.1992, Esteri Ohenoja OULU-F-21543; UNITE UDB07672484). **SWEDEN**, NORRBOTTEN, **Piteå**, Mjöviksmoåsen, 10.1982, Brigitta Öster (UPS-F-740369; UNITE UDB07672488).

ECOLOGY: saprobic on same year's fallen deciduous leaves, most commonly *Populus tremula*, or deciduous litter.

HABITAT: deciduous and mixed woods.

PHENOLOGY: September–October; neotype appeared after first night frost.

DISTRIBUTION: So far confirmed from the Northern Hemisphere, both sides of both North America and Eurasia.

COMMENT. The macroscopic description of *O. umbellifera* is based primarily on the neotype specimen. To spare type material, microscopic observations were augmented by or based entirely on sequence-confirmed conspecific material. A fuller species concept is expected to evolve as *Owingsia* and its species get resolved taxonomically.

Search for a new name for AM-MIN

We began this quest by reviewing descriptions of AM-MIN culled from the cited major workers and those they have quoted, MycoBank and Species Fungorum, appropriate texts, and other sources. FIGURE 7 is a composite plate of some illustrated candidates for AM-MIN from the past, many used in past typification attempts, labelled with year of publication, author, and binomial (where available), all cited in the legend. The plate is arranged in rows to facilitate the discussion around the search for the optimal name. Note that the pleomorphic appearance of the species on this plate resembles that seen on modern photos of AM-MIN (FIG. 1).
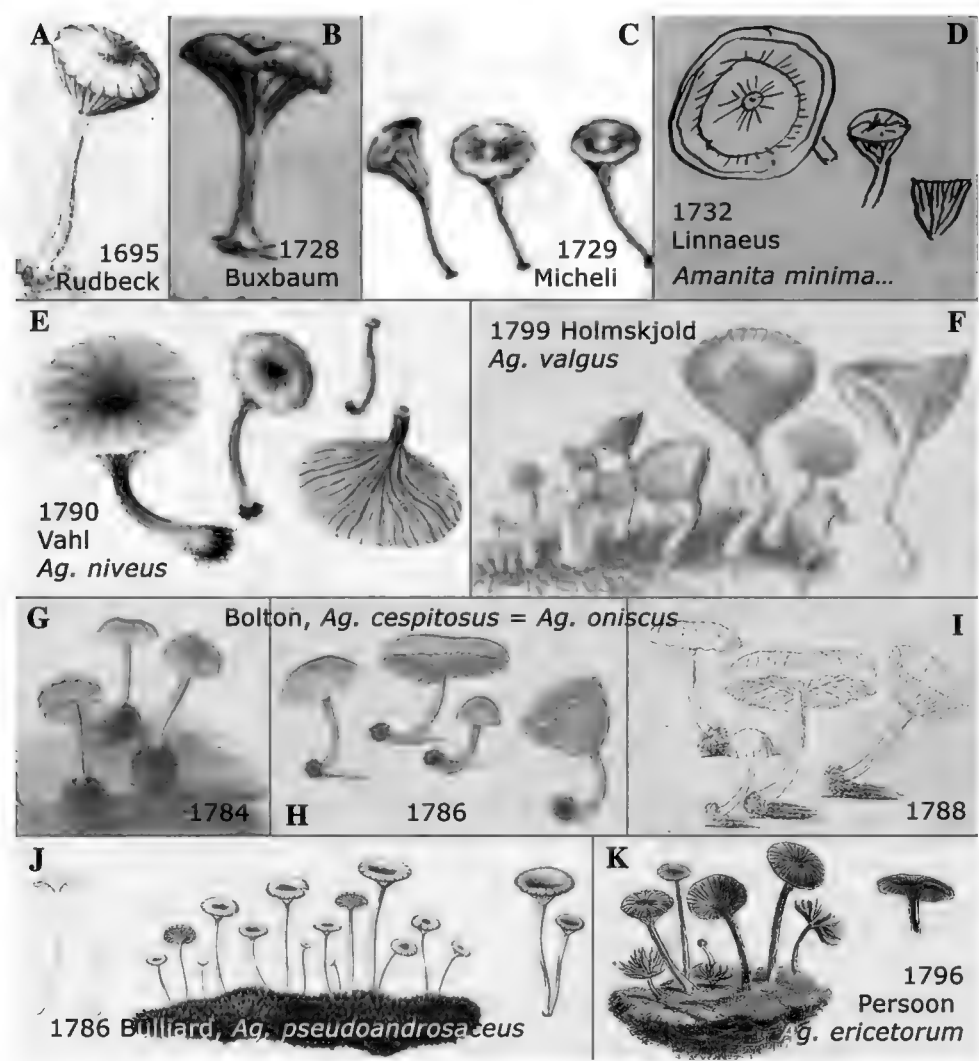
FIG. 7. Composite plate of some real or potential AM-MIN synonyms. Morphological variation akin to that seen on FIG. 1. A. Illustration by Rudbeck (Anfält 1987) of a specimen resembling AM-MIN—same as FIG. 2E, above; no name or description given; B. Illustration by Buxbaum (1728) of a specimen resembling AM-MIN, phrase name given; C. Illustration by Micheli (1729) of a specimen resembling AM-MIN—same as FIG. 2C, above; phrase name given; D. Illustration by Linnaeus (Fries 1913) of a specimen resembling AM-MIN—same as FIG. 2F, above; phrase name given; E. Protologue illustration by Vahl (1790) of *Agaricus niveus*; F. Protologue illustration by Holmskjold (1799) of *Agaricus valgus*; G. Prepublication illustration by Bolton (1784) of *Agaricus cespitosus*, also labelled *Agaricus umbelliferus*; H. Prepublication illustration by Bolton (1786) of *Agaricus*

cespitosus, selected as lectotype for *Agaricus cespitosus* by Voitk (2022); I. Protologue illustration by Bolton (1788) of *Agaricus cespitosus,* selected as lectotype for *Agaricus oniscus* by Voitk (2022); J. Protologue illustration by Bulliard (1786) of *Agaricus pseudoandrosaceus,* the middle group on a moss cushion selected as lectotype for *Agaricus ericetorum* by Redhead & Kuyper (1987); K. Protologue illustration by Persoon (1796) of *Agaricus ericetorum.*

The upper row, Figs 7A–D, predate the use of binomial names; Fig. 7A was drawn but not named or described, and Figs 7B–D were identified by a phrase name. All appeared before 1753, i.e., before the starting-point of valid fungal nomenclature (Art. F.1). Hence, even had they been named, the names would be considered unavailable.

The second row of Fig. 7 shows two taxa with a striking resemblance to Am-min. Figure 7E, *A. niveus,* is an illegitimate name because the epithet was already in use in *Agaricus,* and is thus not available. Figure 7F, *A. valgus,* is unsanctioned, and, therefore, plays no role in the nomenclature of Am-min, so long as a fitting sanctioned name is available.

The third row, Figs 7E–G, show three illustrations by Bolton of *Agaricus cespitosus,* minimally adapted for space. Figures 7G & H come from two volumes in a prepublication folio manuscript, handwritten and hand-painted (Bolton 1784, 1786), each obviously based on a different collection. Fig. 7I shows Bolton's definitive copper plate, used to illustrate his formal printed description of the species (Bolton 1788), obviously based on Fig. 7H. These illustrations were used by Voitk (2022) in a detailed discussion of the synonymy of *A. cespitosus* with *A. oniscus* Fr. nom. sanct., and their conspecificity with Am-min. Namely, 30 years after Bolton described Am-min with the name *A. cespitosus,* Fries (1818) described *A. oniscus,* stating that this new name was to exclude the name *A. cespitosus.* Ordinarily a new name for a legitimate earlier name would be deemed illegitimate as superfluous, but Fries described *A. oniscus* again in his Systema Mycologicum (Fries 1821), giving *A. oniscus* sanctioned priority over *A. cespitosus.* Their synonymy was formalized by declaring the illustration shown in Fig. 7H as lectotype for *A. cespitosus* and Fig. 7I as lectotype for *A. oniscus* (Voitk 2022). Because Fig. 7H was used to make Fig. 7I, the species are homotypic. [We note that due to a typographical error, Voitk (2022) listed the date of publication of Bolton's painting chosen for lectotypification of *A. cespitosus* as 1784, instead of 1786. The correct year appears elsewhere in Voitk's article, and both the description of the image and the citation of source are clear, making the required correction patent.] Although Fries declared that the sanctioned *A. oniscus* was to replace all synonyms (i.e., past, present, and future) for *A. cespitosus,* it takes effect on the

publication date of its protologue, 1818. This gives any sanctioned name for Am-min published before 1818 priority over *A. oniscus*.

The fourth row, Figs 7J & K, are protologue illustrations for the earliest description of Am-min, *A. pseudoandrosaceus* Bull. (Bulliard 1786), and its declared synonym, *A. ericetorum* Pers, described ten years later (Persoon 1796). Again, ordinarily this later synonym would be a considered a superfluous name, but when Fries described the taxon in his 1821 Systema, *A. ericetorum* became sanctioned, taking precedence over Bulliard's name. This ascension to precedence suffered a little transient hiccough because earlier versions of the Code did not extend sanction to names of lichenized fungi. Therefore, when lichenized basidiomycetes were recognized, and Am-min considered one of them, Persoon's name was considered superfluous. Only after the Code extended the same sanctioning rules to names of lichenized fungi, did *A. ericetorum* gain its priority over *A. pseudoandrosaceus*. The effective publication of this sanctioned name was 1796, 22 years before the sanctioned *A. oniscus*, giving *A. ericetorum* priority. Both *A. pseudoandrosaceus* and *A. ericetorum* were lectotypified (Redhead & Kuyper 1987) with the central light-coloured basidiomata on a moss cushion of Fig. 7J, Bulliard's protologue illustration, making them homotypic, thus formalizing Persoon's intended synonymy. One earlier lectotypification (Singer 1961) with a collection made by Persoon was rejected by Redhead & Kuyper because it had not been seen by Bulliard (i.e., could not be part of Bulliard's original material), and was undated, thus lacking evidence that it was part of Persoon's original material. Hence, Redhead & Kuyper's lectotypification is the earliest, and should be followed, according to the Code, now that *A. umbellifer* has been recombined in accordance with its protologue as *Owingsia umbellifera*. In other words, the sanctioned *A. ericetorum* regains its priority (Arts 11.4, F.3) as the basionym for Am-min. So long as *A. pseudoandrosaceus* and *A. ericetorum* are homotypic, attempts to treat them as separate taxa are erroneous (e.g. Singer 1961, Moser 1983), as is the combination of *A. pseudoandrosaceus* to *Mycena* (Bi & al. 1987). Another typification of *A. ericetorum* that should be revised is the epitype designated by Jørgensen & Ryman (1994): specimen 1753 from Fungi Exsiccati Suecici. In the belief that *A. ericetorum* and *A. umbellifer* were synonymous, Jørgensen & Ryman declared it epitype for both. As we see, these names now represent two different species, leaving this typification without standing (Arts 9.18 and 9.20). This paragraph is but a condensed review of a very complex nomenclatural story involving these taxa. Much more detail is

available in Redhead & Kuyper (1987), Redhead & al. (2002), and the many references cited by these authors.

In short, Redhead & Kuyper (1987) have already identified an appropriate name for AM-MIN with a lectotypification, subsequently rejected by Redhead & al. (2002). Should we wish to reconsider it, our task is to ensure that this earliest available name for AM-MIN still remains appropriate and can be reinstated. Persoon cited *A. pseudoandrosaceus* as a synonym, but there must be no conflict between the two protologues, including all associated original material, for his opinion to be valid. There is an obvious colour difference between the two protologue illustrations, Persoon's being much darker than Bulliard's. This discrepancy disappears in their protologue descriptions, making both compatible with each other and with AM-MIN. Bulliard described *A. pseudoandrosaceus* as white to ash grey (gris cendré), occasionally yellowish white. Persoon described *A. ericetorum* as light grey (fragile griseo), and quoted Sibthorpe's (1794) description, lightly dusky (subfusco). The varying references to light grey are compatible with a moist translucent whitish cap, a common appearance of AM-MIN—see FIG. 7G, and a detailed discussion with contemporary photos of this by Voitk (2022). Persoon described the base of the stipe as white, covered with tomentum, and the lamellae as whitish. These descriptions are not congruent with the dark brown basidiomata of Persoon's illustration, suggesting technical problems with rendering accurate colour. Although Persoon did not comment about the colour of his illustration (he may not have seen it at the time of writing), he did mention that the artist had failed to illustrate the shape of the gills adequately, raising some questions about the accuracy of the illustration even before it was painted. We polled five arctic-alpine experts familiar with AM-MIN (Torbjørn Borgen, Jozsef Geml, Gro Gulden, Pierre-Arthur Moreau, Anna Ronikier) about the compatibility of Persoon's illustration with AM-MIN, and not surprisingly, all found the basidiomata dark. Two thought that it was incompatible, without qualification, one specified that the image would be very accurate if it were of lighter colour, and two had no hesitation to accept them as is: one of them volunteered that AM-MIN is so pleomorphic that its spectrum even encompasses the basidiomata on Persoon's image, while the other stated that the green ground cover almost certainly represented a botryoid lichen thallus, and the basidiomal colour should be disregarded entirely, because of the known inaccuracy of hand-painting. Thus, the only problem with the image identified was dark colour, which found no support in the descriptions, and seems best attributed to a

technical artifact. We note that just as Redhead & Kuyper selected only light basidiomata for typification, when choosing a lectotype for *A. oniscus*, Voitk (2022), on encountering a wide variation in colour of hand-painting the same engraving of *A. cespitosus*, specified an unpainted one for typification. Other examples abound. For instance, images of the white *A. porcellaneus* Schaeff. published by Schaeffer (1774), subsequently appear from light to very dark brown in different issues of Bulliard's Herbier de la France, vol 1 (Bulliard 1780).

Our conclusion was that the most likely species intended by both Bulliard and Persoon was AM-MIN, making them synonyms. Although other explanations are possible, support for them seems considerably more tenuous. Therefore, we have no hesitation rejecting the rejection of Redhead & al. of Redhead & Kuyper's lectotypification of both *A. pseudoandrosaceus* and *A. ericetorum*—in other words, reinstating Redhead & Kuyper's lectotypification of both *A. pseudoandrosaceus* and *A. ericetorum* with Bulliard's protologue illustration of *A. pseudoandrosaceus*.

Taxonomy

Lichenomphalia ericetorum (Pers.) Voitk, Thorn & I. Saar, **comb. nov.**   FIGS 1, 8
MB 845595

≡ *Agaricus ericetorum* Pers., Observ. Mycol. 1: 50. 1796 (nom. sanct., Fries, Syst. Mycol. 1: 165. 1821)

≡ *Agaricus pseudoandrosaceus* Bull., Herb. France 6: tab. 276. 1786.

TYPE: **Holotype**: none designated or preserved. **Lectotype** [MBT593068, Redhead & Kuyper 1987], Bulliard tab. 176, 1786, *Agaricus pseudoandrosaceus*. Herbier de la France 6: tab. 276. **Epitype** [**here designated**, MBT10013917], Estonia Tartumaa, Järvselja, 58.2668°N 27.3179°E, 25.08.2016, V. Liiv (TUF120612).

= *Agaricus oniscus* Fr., Observ. Mycol. 2: 209. 1818 (nom. sanct., Fries, Syst. Mycol. 1: 172. 1821)

≡ *Agaricus cespitosus* Bolton, Hist. Fung. Halifax 1: 41, pl. XLI, fig. C. 1788.
MISAPPLICATIONS:

≠ *Agaricus umbellifer* L., Sp. Pl. 2: 1175. 1753.

≡ *Amanita umbellifera* (L.) Roussel, Fl. Calvados: 34. 1796.

≡ *Merulius umbellifer* (L.) With., Arr. Brit. Pl., Edn 3, 4: 147. 1796.

≡ *Omphalia umbellifera* (L.) P. Kumm., Führ. Pilzk.: 107. 1871.

≡ *Omphalina umbellifera* (L.) Quél., Enchir. Fung.: 44. 1886.

≡ *Clitocybe umbellifera* (L.) H.E. Bigelow, Can. J. Bot. 37: 773. 1959.

≡ *Lichenomphalia umbellifera* (L.) Redhead & al., Mycotaxon 83: 38. 2002.

Fɪɢ. 8. Epitype of *Lichenomphalia ericetorum* (TUF120612) in situ. Photo: Vello Liiv.

Cᴀᴘsᴜʟᴀʀ ᴇᴘɪᴛʏᴘᴇ ᴅᴇsᴄʀɪᴘᴛɪᴏɴ (Fɪɢ. 8)

Mᴀᴄʀᴏsᴄᴏᴘɪᴄ: Basidiomata omphalinoid. Pileus up to 20 mm, low convex to plane, centre often umbilicate, smooth, margin crenulate, translucently striate, pale yellow. Lamellae deeply decurrent, sometimes forked, distant, concolorous with pileus or paler. Stipe 10–20 × 1–2 mm, smooth, dry, yellowish.

Mɪᴄʀᴏsᴄᴏᴘɪᴄ: Basidiospores 7.5–12 × (4.8–)5.3–8.8 μm, mean 9.9 × 6.8 8 ± 1.4 μm, Q = 1.3–1.6, mean 1.4 ± 0.1; hyaline, inamyloid, broadly ellipsoid to ellipsoid. Basidia 30–32 × 9–11 mm, 4-spored. Cystidia and clamp connections absent.

Nᴏᴛᴇ: Fɪɢ. 1 and its legend discussion give an overview of the pleomorphic spectrum of the species.

Discussion

Completion of this quest was only made possible by unprecedented good fortune. Our first anticipated impediment was the nature of early species descriptions: often very brief, somewhat vague, lacking important information, inconsistent, and seemingly based on what are often considered several species.

With respect to *A. umbellifer*, Linnaeus surprised us with a consistent cluster of solid characters that formed a very clear picture of the species he had in mind. A robust species concept makes comparison for fit to a known species much easier, but this particular step of the enquiry was greatly aided by Redhead & Kuyper (1987), who had already documented the major conflicts arising from trying to apply *A. umbellifer* to Am-min.

The hubris of requiring that we find an unequivocal fit for *A. umbellifer* was not lost on us, and we retained healthy skepticism about our ability to meet it. We set this condition knowingly at the outset, in the belief that nomenclatural stability would be served only if *Agaricus umbellifer* were securely fixed by concinnous typification. A commodious match for the epithet would: a) be a solid test of whatever species concept we could develop from Linnaeus's writings; b) avoid orphaning a name in use for over a quarter millennium; and c) justify the search for a more fitting name for Am-min. Success would require familiarity with Lapland mycota, something not possible from a desk, bookshelf, or armchair.

This step was realized, against our own expectations, partly thanks to the limpid picture painted by Linnaeus's descriptions. Just as Linnaeus immediately recognized the species on Öland, some nine years after seeing it in Lapland, the senior author, AV, once armed with a clear vision of Linnaeus's concept and its Lapland location, immediately recalled a similar species he had encountered in Lapland 14 years earlier. This find was not the result of foresight or clever experimental design, but mere fortuitous happenstance. Because one of his sons lived several years in Norwegian Lapland, AV had made multiple visits to the area, exploring the region between Finnsnes and Nordkapp, east to nearby Finland. In addition to the iconic Am-min, documented by both Rudbeck and Linnaeus, he encountered several species typical of the habitat, some of which have been reported elsewhere [*Gomphidius roseus* (Fr.) Oudem. (Aime & Voitk 2014); *Cantharellus cibarius* Fr. (Thorn & al. 2017); *Chromosera lilacina* (P. Karst.) Vizzini & Ercole (Voitk & Voitk 2020); *Arrhenia philonotis* (Lasch) Redhead & al. (Voitk & al. 2022)]. Encountering *O. umbellifera* (Fig. 3) in 2006 made it clear why it is not collected more frequently. For over a week AV had taken the same forest path daily to explore the barren higher land around the tree line, without seeing this species. One morning, after the first night frost, large numbers became evident on fallen leaves along the forest trail, where none had been noted the day before. The following day, when the photo for Fig. 3A was taken, very few were left, and on the third day it was difficult to find any sign of their passing. Scopoli (1772) confirmed that this is not a

chance observation, noting "brevis vita" as one of characters of *A. umbellifer*. In other words, even if the species is common and ubiquitous, it can easily escape detection because of its unison fruiting within a very narrow timespan and its capriciously ephemeral basidiomata.

Finding an epiphyllic species on fallen leaves of *P. tremula*, appropriately another Linnaeus species, around 70°N may seem unexpected, but Lapland is warmed by the tail end of the Gulf Stream (Voitk 2021), enabling substantial coniferous and deciduous forests to thrive north of the Arctic Circle. As glaciation receded, the psychrophilic *P. tremula* passed through Fennoscandia and the Baltic Sea islands to traverse Lapland, and reach Murmansk and beyond. With it came *O. umbellifera*, served up to us by serendipity on the aspen-lined Lapland trail. This species, confirmed to be prevalent in Lapland by our sequences (FIG. 6), accommodates Linnaeus's protologue for *A. umbellifer* better than any earlier attempt, reconciling even the flat cap that had remained a problem in the past. Applying *A. umbellifer* to a fitting species permits Fries's apportionment of Micheli's epiphyllic fungus to remain intact, also a more fitting result.

In their deliberations, Redhead and colleagues suspected that, just as Linnaeus's descriptions of *A. umbellifer*, descriptions of *A. pseudoandrosaceus* and *A. ericetorum* also incorporated elements from more than one species. Recent technological advances, like molecular studies, confirm this as very likely: the ability to identify evolutionary pathways has uncovered many complexes of cryptic species hiding under one name. Some of the species in *Owingsia*, all identified as *Marasmius epiphyllus*, may prove to be one such example. The lack of unanimity among our expert panel confirms that Bulliard's and Persoon's illustrations are a bit shy of ideal, even if they lack such major conflicts like long vs normal stipe and epiphyllic vs non-epiphyllic ecology. Many, if not most old descriptions can be assumed to contain elements of more than one species, which becomes a general problem for future nomenclatural and taxonomic work. If the conflict trigger becomes too sensitive, options may be to disregard all old descriptions as inaccurate, vague, or otherwise imperfect, and treat each species as new. Alternately, all such questions may be sent to binding resolution by motions to conserve one name against another. Neither approach seems attractive. Detailed review of the original material convinced us that, despite some slight aberrations possibly due to technical factors and possibly to unpreventable inclusion of similar species, AM-MIN was by far the most likely species intended by *A. pseudoandrosaceus* and *A. ericetorum*.

Were we to reject the synonymy of *A. ericetorum* and *A. pseudoandrosaceus*, the earliest valid names for Am-min would become *A. cespitosus*, with a closer fit to Am-min than has *A. ericetorum*, and replaced by the sanctioned *A. oniscus*, whose synonymy with *A. cespitosus* seems beyond challenge according to current rules of nomenclature. However, such rejection would be based on very small inconsistencies, difficult to defend—a weak foundation inviting future challenges. Nomenclature, lacking an inherent need for change, should strive to follow the "one fungus = one name" (Taylor 2011) principle that makes infungible fungal epithets a marked improvement on common names. However, we lack the enthusiasm of some onomasts for nomenclatural stability at all cost, and believe that there should be no hesitation to correct application of names producing serious conflict with the original protologue material. Stability in such considerations should not confuse "established custom" with personal preference, which only moves instability to the future. In these times of great phylogenetic discovery, names change almost daily. Correction of a few discordant nomenclatural misapplications accounts for a negligible proportion of these changes, almost all of which come from changed taxonomic concepts. Stability in taxonomy is an unrealistic and unattainable goal, because taxonomy is evidence-based ranking, inherently changing as new knowledge accrues.

An interesting observation during this work was that reassessment of how we interpret citations may be helpful; automatically assigning synonymy to every citation may not reflect the authors' intent. Consider that Linnaeus stated five times that the pileus of *A. umbellifer* is flat, yet cited illustrations of one species with a hemispherical and another with a subconic pileus. To maintain that Linnaeus cited these as synonyms, something he did not claim, presupposes that either he did not know the meaning of the "flat", or was blind—yet it has been done. [In contrast, consider that although Fries stated in no uncertain terms that he introduced *A. oniscus* specifically as a synonym to exclude *A. cespitosus*, many workers elected to ignore Fries's stated intent, and applied *A. oniscus* to morphologically different taxa, totally unrelated to the original material supporting the epithet (Voitk 2022).]

Fries's handling of *A. umbellifer* provides a further opportunity to examine interpretation of citations. Fries (1825) first mentioned *A. umbellifer* in a review of the flora around his home, Femsjö, stating that Linnaeus's synonym was surely restored, thus emphasizing that the species concept under discussion is that of Linnaeus. Fries did not describe the species, but instead cited Pollich (1777), saying the latter provided a good description of it. Indeed, Pollich cited three of Linnaeus's descriptions as well as the Micheli description cited by Linnaeus,

discussed above, and another description by Scopoli (1772). All described a small white long-stemmed basidioma, to which Pollich added a description of the pileus (white, convex, becoming plane) and lamellae (white, initially arising evenly, then descending slightly to become subdecurrent at the stem). Pollich's description augmented by the descriptions he cited, is congruent with the species concept of Linnaeus and matches *O. umbellifera*. Fries next sanctioned *Agaricus umbellifer*. Again, Fries did not describe the taxon himself. The heading, "*A. umbelliferus* Linn.", is a de facto citation of Linnaeus's description (Linnaeus 1753), to which Fries added a citation of his first treatment of the name, described above, which provided various citations of descriptions fitting that of Linnaeus. Thereafter followed three citations of works applying *A. umbellifer* to a short-stemmed (stipite brevi) species: Wahlenberg (1826), Sommerfeldt (1826), and Fries's treatment of *A. ericetorum* (Fries 1821).

It is highly unlikely that anyone, let alone the father of Friesian taxonomy, would proffer species with such discordant characters as long and short stems as conspecific. The only logical conclusion we could draw is that the reason to mention treatments of a short-stemmed species as *A. umbellifer* was an attempt to alert the reader to some recent misapplications of the Linnaean name. We know that Fries had no difficulty indicating synonymy, when such was his intent; perhaps the reason he did not declare the last three citations as conspecific was that he did not believe they were. That this was Fries's intent finds strong support from his citation of his own description of *A. ericetorum* (Fries 1821). He very specifically indicated that he did **not** cite it as a synonym, by preceding the citation with "V." (videre = see, view), an invitation to the reader to view (in the sense of compare and contrast) the description to judge its aptness. Surely, he was not inviting the reader to compare *A. ericetorum* to the *A. umbellifer* of Linnaeus? He knew these were different species characterized by markedly different sized stipes and markedly different substrate preferences. Rather, he invited a comparison of *A. ericetorum* to the species to which both Wahlenberg and Sommerfeldt had misapplied the epithet *umbellifer*, an obvious suggestion that *A. ericetorum* may be a better fit for those species than (the misapplied) *A. umbellifer*. Therefore, it is not surprising to learn that the descriptions of Wahlenberg and Sommerfeldt do resemble *A. ericetorum* far better than *A. umbellifer*. Failure to understand this has caused some regrettable confusion that Fries sought to synonymize *A. ericetorum* with *A. umbellifer*.

We dealt with two species concepts described over 200 years ago, both interpreted and reinterpreted with much passionate debate over the years. The original names of both have been misapplied to other species, and both species

have had other names misapplied to them. Whether achieved by luck or design, finding names to apply to both species that fit the original material without conflict, reflect their authors' intent, and observe current rules of nomenclature is gratifying.

Epilogue

The Preamble to the Code states, "The object of the rules is to put the nomenclature of the past into order and to provide for that of the future … The only proper [reason] for changing a name [is] … a more profound knowledge of the facts resulting from adequate taxonomic study …" We believe our solution embodies this object, while settling a longstanding problem. The major attraction of this solution is its pleasing concinnity: the application of *A. umbellifer* will be the first since Linnaeus's description that will fit his concept without conflict, and the familiar *A. ericetorum* will be reinstated, hopefully to continue the stability it enjoyed earlier. Names fitting with their original material without conflict are unlikely to need change, ensuring future stability in return for minimal transient discomfort. We wish to leave stable names to our colleagues of tomorrow, rather than ask them to accept ill-fitting names because for a brief period in the long history of these names we may have become comfortably accustomed to one version of their misapplication.

Moreau (Université de Lille, France), as formal reviewers, made major contributions to a revised and improved content; the Editor-in-Chief, Lorelei Norvell, is thanked for pertinent suggestions, as well as help with buffing textual asperity to produce a more accurate and readable final product; an especial thanks is owed to the Nomenclatural Editor, Shaun Pennycook, who worked beyond reasonable editorial limits to minimize the authors' deficiency in this area. This is the second nomenclatural study prompted by a review of sphagnicolous arrhenias (Voitk & al. 2022), and understandably, in addition to the assistance acknowledged here, we are indebted to all whose efforts were gratefully documented in the original study and its first nomenclatural derivative (Voitk 2022), which spawned the current investigation.

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New species of *Catenularia and Fuscocatenula* from Xishuangbanna, China

Jing-Wen Liu[1], Ya-Fen Hu[1], Ru-Qiang Cui[1], Rafael F. Castañeda-Ruíz[2],
Zhao-Huan Xu[1], Jian Ma[1]*

[1] *College of Agronomy, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, China*
[2] *Instituto de Investigaciones Fundamentales en Agricultura Tropical 'Alejandro de Humboldt'*
    (INIFAT), Académico Titular de la "Academia de Ciencias de Cuba",
    Calle 1 Esq. 2, Santiago de Las Vegas, C. Habana, Cuba, C.P. 17200

* Correspondence to: *majian821210@163.com; jxaumj@126.com*

Abstract—Three new anamorphic fungi, *Catenularia yunnanensis, Fuscocatenula nabanhensis* and *F. chinensis*, are described and illustrated from specimens collected on dead branches of unidentified plants in Xishuangbanna, Yunnan Province, China. *Catenularia yunnanensis* is characterized by its acrogenous, basocatenate, aseptate, rounded-obconic, smooth conidia with 2–3 blunt corners at the distal end when viewed from above. *Fuscocatenula nabanhensis* is distinguished by its basocatenate, aseptate, cylindrical to slightly cuneiform, smooth conidia with protracted maturation and round outline. *Fuscocatenula chinensis* is denoted by its basocatenate, aseptate, obovoid, smooth conidia with round outline. The three species are described, illustrated, and compared with similar taxa. A key to species of *Fuscocatenula* is provided.

Key words—asexual fungi, *Chaetosphaeriaceae*, hyphomycetes, conidial fungi, taxonomy

Introduction

Catenularia Grove was erected with *Catenularia simplex* Grove [= *C. cupulifera* (Berk. & Broome) Réblová & A.N. Mill.] as the type species (Hughes 1965, Ellis 1971, Réblová & al. 2021), and was mainly characterized by macronematous, mononematous conidiophores, and integrated, terminal, monophialidic, percurrently extending conidiogenous cells with a funnel shaped collarette at

the apex, and basocatenate, aseptate, cuneiform or rounded-obconic conidia with several blunt corners at the distal end when viewed from above (Hughes 1965, Ellis 1971, Réblová & al. 2021). Hughes (1965) reviewed the history of *Catenularia*, and excluded ten species from the genus. Subsequently, Réblová & al. (2021) evaluated the generic concept of *Catenularia* and its relationships with morphologically similar fungi based on molecular and morphological data, accepted eleven species, and proposed the segregate genus *Fuscocatenula* Réblová & A.N. Mill. for *Chaetosphaeria submersa* Z.L. Luo & al. (type) and *Catenularia variegata* H.H. Li & X.G. Zhang, readily distinguishable from typical *Chaetosphaeria* and *Catenularia* by pigmented conidia with protracted maturation and round outline.

Xishuangbanna Dai Autonomous Prefecture, located in the southwestern part of Yunnan Province, China, is a famous biodiversity hotspot (Cao & Zhang 1997). It lies in 21.17–22.67°N 99.92–101.83°E and has a mountainous topography with remarkable seasonal climate variations. Its mean annual minimum and maximum temperatures are 19.3–23.9°C, and the total annual precipitation is about 1493 mm. Such favorable conditions maintain large areas of tropical rain forest which contain rich biodiversity. During our mycological surveys in the forest ecosystems of this region, three hyphomycetes with morphological features typical of *Catenularia* (Grove 1886) and *Fuscocatenula* (Réblová & al. 2021) were collected on dead branches. However, these collections are morphologically easily distinguishable from the currently accepted species of these genera and are proposed here as new taxa.

Materials & methods

Samples of dead branches were collected from humid environments and river bank in the forest ecosystems of Xishuangbanna, and placed in Ziploc™ bags for transporting to the laboratory, where they were processed, and examined as described by Ma & al. (2011). Fungi were mounted in a drop of lactic acid on microscope slides, examined and photographed with an Olympus microscope (model BX 53) under a 100× (oil immersion) objective at the same background and scale. Adobe Photoshop 7.0 was used for image processing to assemble photographs into images. Single-spore isolations were made on potato dextrose agar (PDA) following Goh (1999) but were unsuccessful after several attempts. The specimens are deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, Jiangxi, China (HJAUP).

Taxonomy

Catenularia yunnanensis Jing W. Liu, Jian Ma & R.F. Castañeda, **sp. nov.**   Fig. 1

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Differs from *Catenularia catenulata* by its smaller conidia with 2–3 corners; from *C. cupulifera*, *C. minor*, and *C. novae-zelandiae* by its smaller conidia with 2–3 corners and by the absence of capitate hyphae; and from *C. cubensis* by its wider conidia with 2–3 corners and by the absence of capitate hyphae.

TYPE: China, Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, the Xishuangbanna Tropical Botanical Garden, on dead branches of an unidentified broadleaf tree, 9 July 2021, J.W. Liu (**Holotype**, HJAUP M0086-2).

ETYMOLOGY: refers to Yunnan province where the type was collected.

FIG. 1. *Catenularia yunnanensis* (holotype, HJAUP M0086-2). Conidiophores, conidiogenous cells, and conidia.

COLONIES on natural substratum effuse, brown, hairy. Mycelium superficial and immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae. Capitate hyphae absent. CONIDIOPHORES macronematous, mononematous, simple, erect, straight or flexuous, cylindrical, smooth, 1–5-septate, brown, paler towards to the apex, thick-walled, 135–175 × 4.5–5.5 μm. CONIDIOGENOUS CELLS monophialidic, integrated, terminal, cylindrical, brown to pale brown, percurrently extending, with a distinct, funnel-shaped collarette at the apex, 25–40 μm long, 4–6 μm diam. CONIDIA basocatenate, up to 11 or more, aseptate, rounded-obconic, smooth, truncate at the base, with 2–3 blunt corners at the distal end when viewed from above, 7.5–8 μm long, 7–8 μm diam. at the apical end, 1.5–2.5 μm diam. at the truncate base.

COMMENTS—Among the known species of *Catenularia*, *C. yunnanensis* is most similar to *C. cubensis* Hol.-Jech., *C. cupulifera*, *C. novae-zelandiae* (S. Hughes & Shoemaker) Réblová & A.N. Mill., *C. catenulata* (Z.L. Luo & al.) Réblová & A.N. Mill., and *C. minor* (Hol.-Jech.) Réblová & A.N. Mill. in conidial shape (Holubová-Jechová 1982, Réblová & al. 2021). However, *C. catenulata* differs by its turbinate-triangular, larger conidia (13–15 × 12–14 μm) with 3–4 corners (Luo & al. 2019, Réblová & al. 2021); *C. cupulifera* by its cuneiform, larger conidia (10.5–13.5 × 7–9.5 μm) with (3–)4(–5) corners (Réblová & al. 2021); *C. cubensis* by its rounded-obconic to broadly obovoid, narrower conidia (3.5–5.5 μm wide) with 3 corners (Holubová-Jechová1982, Réblová & al. 2021); *C. minor* by its cuneiform to rounded-obconic to obtriangular, larger conidia (7.5–10.5×6.5–11.5 μm) with 3–5 corners (Holubová-Jechová 1983, Réblová & al. 2021); and *C. novae-zelandiae* by its larger conidia (11.5–17.5 × 14.5–18.5 μm) with 4–5 corners (Hughes 1965, Réblová & al. 2021). In addition, *C. cubensis*, *C. cupulifera*, *C. novae-zelandiae*, and *C. minor* have capitate hyphae, which are not found in *C. catenulata* and *C. yunnanensis*.

Fuscocatenula nabanhensis Jing W. Liu, Jian Ma & R.F. Castañeda, **sp. nov.**   FIG. 2
IF 559609

Differs from *Fuscocatenula submersa* and *F. variegata* by its cylindrical to slightly cuneiform, smaller conidia.

TYPE: China, Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, the Nabanhe National Nature Reserve, on dead branches of an unidentified broadleaf tree, 9 July 2021, J.W. Liu (**Holotype**, HJAUP M2061).

ETYMOLOGY: Latin, *nabanhensis* referring to the place, Nabanhe, where the fungus was collected.

Fɪɢ. 2. *Fuscocatenula nabanhensis* (holotype, HJAUP M2061). A–C. Conidiophores, conidiogenous cells, and conidia; D. Conidia.

Coʟonɪᴇs on natural substratum effuse, brown, hairy. Mycelium superficial and immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae. Capitate hyphae absent. Conɪᴅɪophoʀᴇs macronematous, mononematous, simple, erect, straight or flexuous, cylindrical, smooth, 2–6-septate, brown, paler towards to the apex, thick-walled, 55–130 × 3.5–4 µm. Conɪᴅɪoɢᴇnous cᴇʟʟs monophialidic, integrated, terminal, cylindrical,

pale brown, percurrently extending, with a distinct, funnel-shaped collarette at the apex, 20–30 μm long, 3.5–5 μm diam. Conidia basocatenate, up to 18 or more, aseptate, cylindrical to slightly cuneiform, smooth, 5–6 × 3–3.5 μm, rounded at apex, truncate at base, hyaline while young, becoming pale brown at mature.

Comments—Réblová & al. (2021) proposed *Fuscocatenula*, and to date only *F. submersa* (Z.L. Luo & al.) Réblová & A.N. Mill. and *F. variegata* (H.H. Li & X.G. Zhang) Réblová & A.N. Mill. have been included. *Fuscocatenula submersa* differs from *F. nabanhensis* by its cuneiform, larger conidia (21–27 × 12–14 μm), and longer and wider conidiophores (380–596 × 15–21 μm) (Luo & al. 2019, Réblová & al. 2021); and *F. variegata* by its obovoid, larger conidia (8.5–11 × 5.5–7.5 μm) and longer and wider conidiophores (150–270 × 4.5–8 μm) (Li & al. 2017, Réblová & al. 2021).

Fuscocatenula chinensis Jing W. Liu, Jian Ma & R.F. Castañeda, **sp. nov.**   Fig. 3
IF 559610

> Differs from *Fuscocatenula submersa* by its obovoid, smaller conidia and shorter and narrower conidiophores; and from *F. variegata* by its longer and narrower conidia and longer conidiophores.

> Type: China, Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, the Nabanhe National Nature Reserve, on decaying twigs of an unidentified broadleaf tree, 9 July 2021, J.W. Liu (**Holotype**, HJAUP M0041).

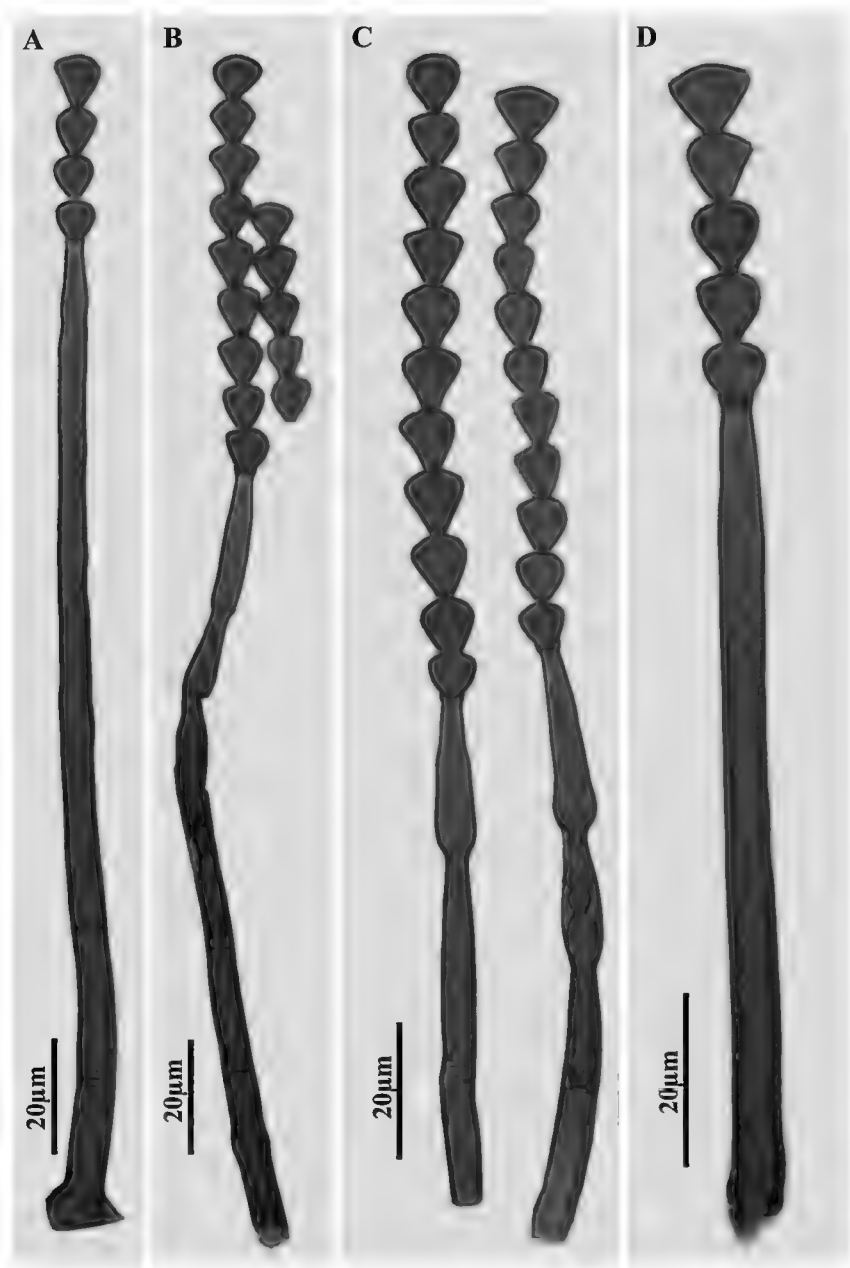> Etymology: refers to China, in which the fungus was collected.

Colonies on natural substratum effuse, brown, hairy. Mycelium superficial and immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae. Capitate hyphae absent. Conidiophores macronematous, mononematous, simple, erect, straight or flexuous, smooth, 6–11-septate, brown to dark brown, paler towards to the apex, thick-walled, 275–360 × 6.5–8 μm. Conidiogenous cells monophialidic, integrated, terminal, cylindrical, brown to pale brown, percurrently extending, with a distinct, funnel-shaped collarette at the apex, 44–75 μm long, 5–7 μm diam. Conidia basocatenate, aseptate, obovoid, smooth, 11–12 × 5–5.5 μm, rounded at apex, truncate at base, with a protracted maturation, the conidia are hyaline and only later become pale brown, while still attached in a chain, sometimes the chain formed by hyaline conidia with only several mature pigmented conidia.
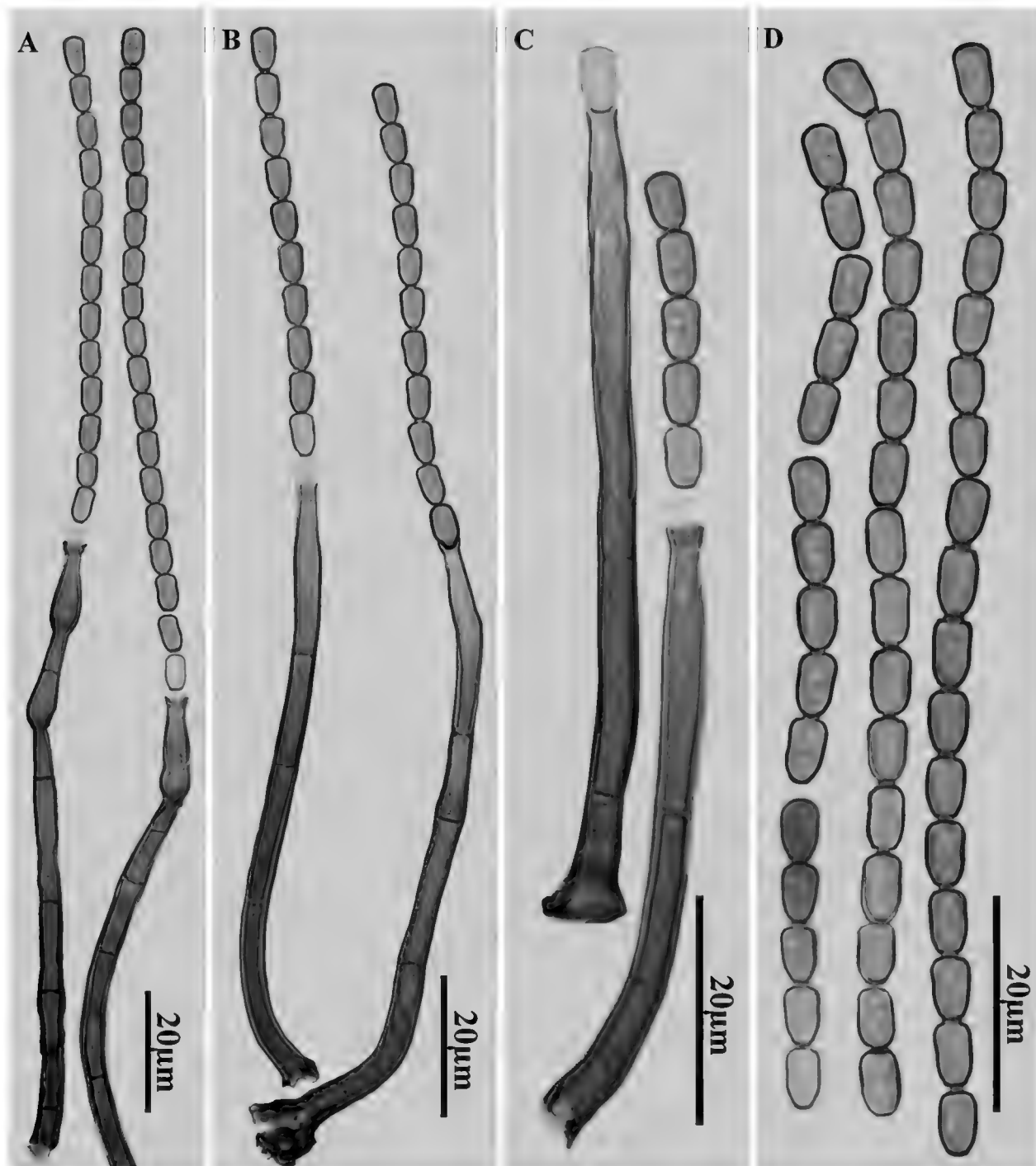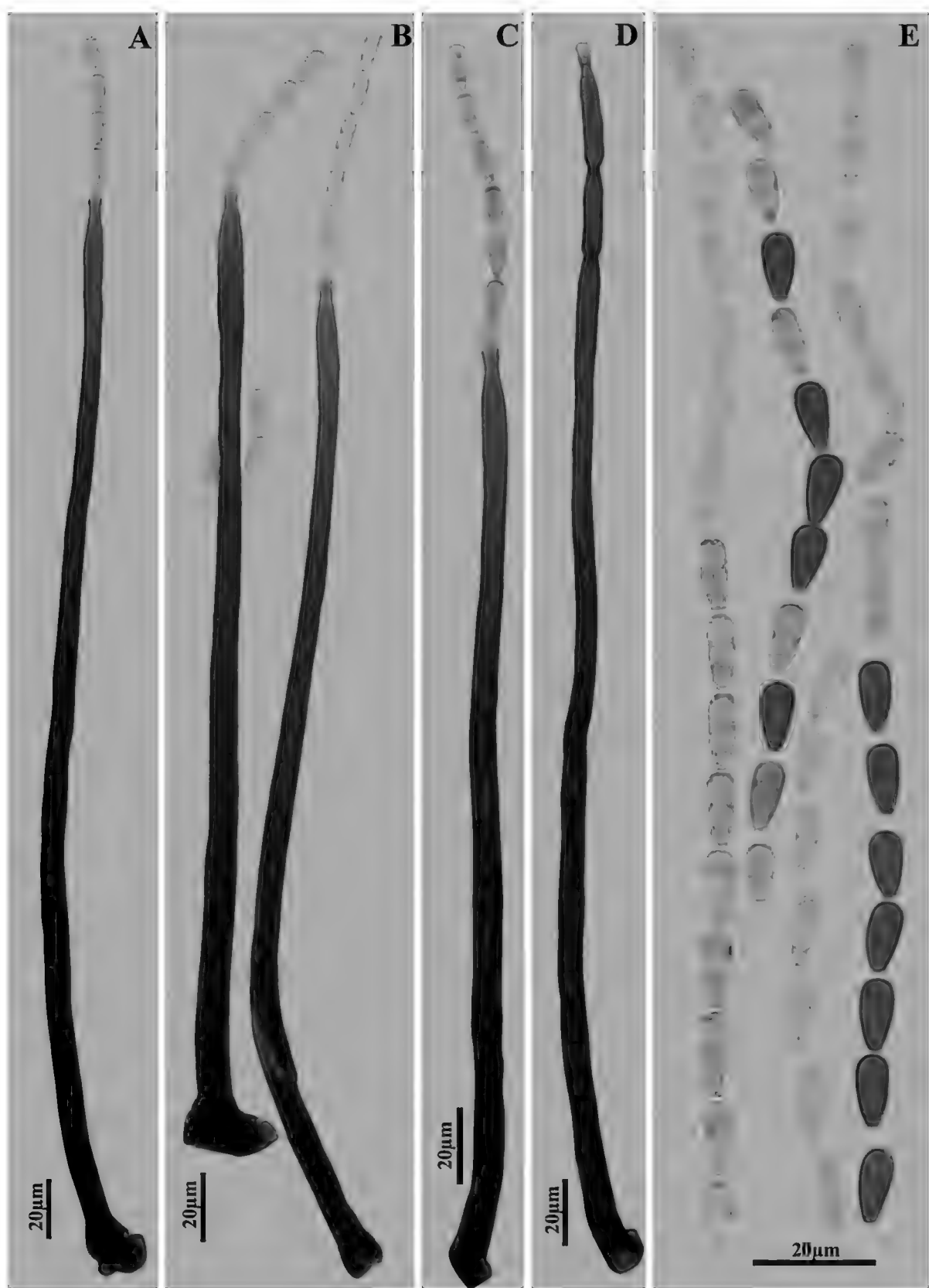
FIG. 3. *Fuscocatenula chinensis* (holotype, HJAUP M0041). A–D. Conidiophores, conidiogenous cells, and conidia; E. Conidia. (the hyaline and pale-brown color assumed a greenish cast during processing).

COMMENTS—*Fuscocatenula chinensis* is morphologically similar to *F. submersa* and *F. variegata*, but *F. submersa* differs by its cuneiform, larger conidia (21–27 × 12–14 μm), and longer and wider conidiophores (380–596 × 15– 21 μm) (Luo & al. 2019, Réblová & al. 2021); and *F. variegata* by its shorter and wider conidia (8.5–11 × 5.5–7.5 μm) and shorter conidiophores (150–270 μm long) (Li & al. 2017, Réblová & al. 2021). In addition, *F. chinensis* differs from *F. nabanhensis*, which has cylindrical to slightly cuneiform, smaller conidia and shorter and narrower conidiophores.

Key to *Fuscocatenula* species

1. Conidia cylindrical to slightly cuneiform, 5–6 × 3–3.5 μm .... *F. nabanhensis*
1. Conidia obovoid or cuneiform, ≥8.5 × 5 μm 2
2. Conidia cuneiform, 21–27 × 12–14 μm .... *F. submersa*
2. Conidia obovoid, ≤12 × 7.5 μm 3
3. Conidia 8.5–11 × 5.5–7.5 μm; conidiophores 10–12-septate, 150–270 × 4.5–8 μm
.... *F. variegata*
3. Conidia 11–12 × 5–5.5 μm; conidiophores 6–11-septate, 275–360 × 6.5–8 μm
.... *F. chinensis*

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Distocercospora curvulata sp. nov. from northern India

SANJEET KUMAR VERMA, SANJAY YADAV, RAGHVENDRA SINGH[*]

Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, U.P., India 221005

[*]CORRESPONDENCE TO: *drsinghtaxon@gmail.com, singhr.bot@bhu.ac.in*

ABSTRACT— A new species of asexual foliicolous fungus, *Distocercospora curvulata*, discovered on living leaves of *Causonis trifolia* from Uttarakhand, India, is described and illustrated.

KEY WORDS— hyphomycetes, *Mycosphaerellaceae*, phytopathogenic fungi, taxonomy

Introduction

Cercospora is the largest genus of asexual fungi which is commonly referred as cercosporoid fungi belonging to *Mycosphaerellaceae* (*Mycosphaerellales, Ascomycota*). It represents a large group of leaf spotting, plant pathogenic species, causing diseases on a wide range of hosts (Crous & Braun 2003; Braun & al. 2013, 2016). The distoseptate nature of conidia is exhibited by *Distocercospora* N. Pons & B. Sutton and *Distocercosporaster* Videira & al. within the cercosporoid complex (Braun & al. 2014, Videira & al. 2017). Morphologically, *Distocercospora* is characterized by plant pathogenic species, stromata lacking to well-developed; conidiophores macronematous, fasciculate, simple to strongly branched, septate; conidiogenous cells integrated, terminal to intercalary, loci protuberant, thickened and darkened; conidia solitary to rarely concatenate, mostly obclavate to cylindrical, single to several transverse distosepta or a mixture of eu- and distosepta, hila thickened and darkened (Braun & al. 2014, Videira & al. 2017). *Distocercospora* differs from *Distocercosporaster*, which has stromata composed of subhyaline to brown

swollen hyphal cells, short conidiophores, rim-like and distinctly thickened conidiogenous loci on terminal conidiogenous cells, and frequently catenate conidia (Videira & al. 2017).

A survey of plant pathogenic fungi for systematic exploration of conidial fungi was carried out in Uttarakhand and a new fungal species was collected on living leaves of *Causonis trifolia*. Morphologically, this fungus resembles *Distocercospora*. A number of conidial fungi have been described from India (Kumar & Singh 2015a,b, 2016; Singh & Kamal 2011; Awasthi & al. 2016; Kumar & al. 2012a,b, 2018; Kushwaha & al. 2020; Singh & al. 2011, 2012, 2013a,b, 2014a,b, 2019, 2020) suggesting that the diversity of such fungi is still insufficiently known in this region.

Materials & methods

In September 2019, an infected leaves of *Causonis trifolia* were collected from Valley of Flowers National Park, Uttarakhand during field survey conducted for collections of phytopathogenic fungal samples. Fungal samples were kept in sterilized polythene bags. The collected samples were processed by following the standard techniques (Hawksworth 1974, Savile 1962). Slides for microscopic examination were prepared by scraping taken from infected area of freshly collected materials and mounted in clear lacto-phenol cotton blue mixture. The microphotographs of fungal propagules were taken by using Magnus camera (MIPS CMOS) attached with an Olympus trinocular compound microscope (CH20i-TR). The detailed morphological observations were carried out at different magnifications (450× and 1000×). Measurements were made of 20 each morphological features. The holotype specimen was deposited in the Ajrekar Mycological Herbarium, Ajrekar Research Institute, Pune, India (AMH) and the isotype material was retained in the Mycological Herbarium, Department of Botany, Banaras Hindu University, Varanasi, U.P., India (MH-BHU).

Taxonomy

Distocercospora curvulata S.K. Verma, Sanj. Yadav & Raghv. Singh, **sp. nov.**

FIGS 1–3

MB 838244

Differs from other *Distocercospora* spp. by the presence of euseptation along with distoseptation in conidia.

TYPE: India, Uttarakhand, Valley of Flowers National Park, 30.7333°N 79.6333°E, on living leaves of *Causonis trifolia* (L.) Mabb. & J. Wen (*Vitaceae*), 23 September 2019, coll.

Raghvendra Singh (**Holotype**, AMH 10308; **isotype**, MH-BHU 35).

Etymology: Latin, referred to the curved conidia.

Fig. 1. *Distocercospora curvulata* on *Causonis trifolia*. a. Host plant in natural habitat; b. Symptom on lower surface of leaf. Scale bar: b = 5 mm.

Infection spots hypogenous, discrete, initially whitish, later on become brown to blackish brown, surrounded by whitish margin, circular, 3–6 mm. Colonies hypophyllous, effuse, velvety, initially whitish, late on turned to brown to dark blackish brown surrounded by white margin. Mycelium internal, composed of branched, septate, smooth, thick-walled, olivaceous to light brown hyphae. Stromata well-developed, internal to erumpent, pseudoparenchymatous, brown to dark blackish brown, 12–46 × 8–30 μm diam. Conidiophores macronematous, densely fasciculate, arising from stromata, cylindrical, erect to procumbent, straight to flexuous, geniculate, unbranched, 1–7-septate, thick-walled, olivaceous to light brown to mid brown, lighter towards at apex, (22–)34–92(–103) × 3.5–7 μm. Conidiogenous cells integrated, terminal to intercalary, mono to polyblastic, proliferation sympodial, scars conspicuously protuberant, thickened and darkened, 1.5–2 μm. Conidia solitary, dry, acropleurogenous, acicular, narrowly obclavate to subcylindrical, curved, rarely straight, 0–5-distoseptate and 0–3-euseptate, sometimes constricted at the septa, thin-walled, smooth, apex obtuse, base obconically rounded, light olivaceous-brown to light brown, 22–80(–95) × 2–7 μm, hilum sometimes projecting, thickened and darkened, 1.5–2 μm wide.
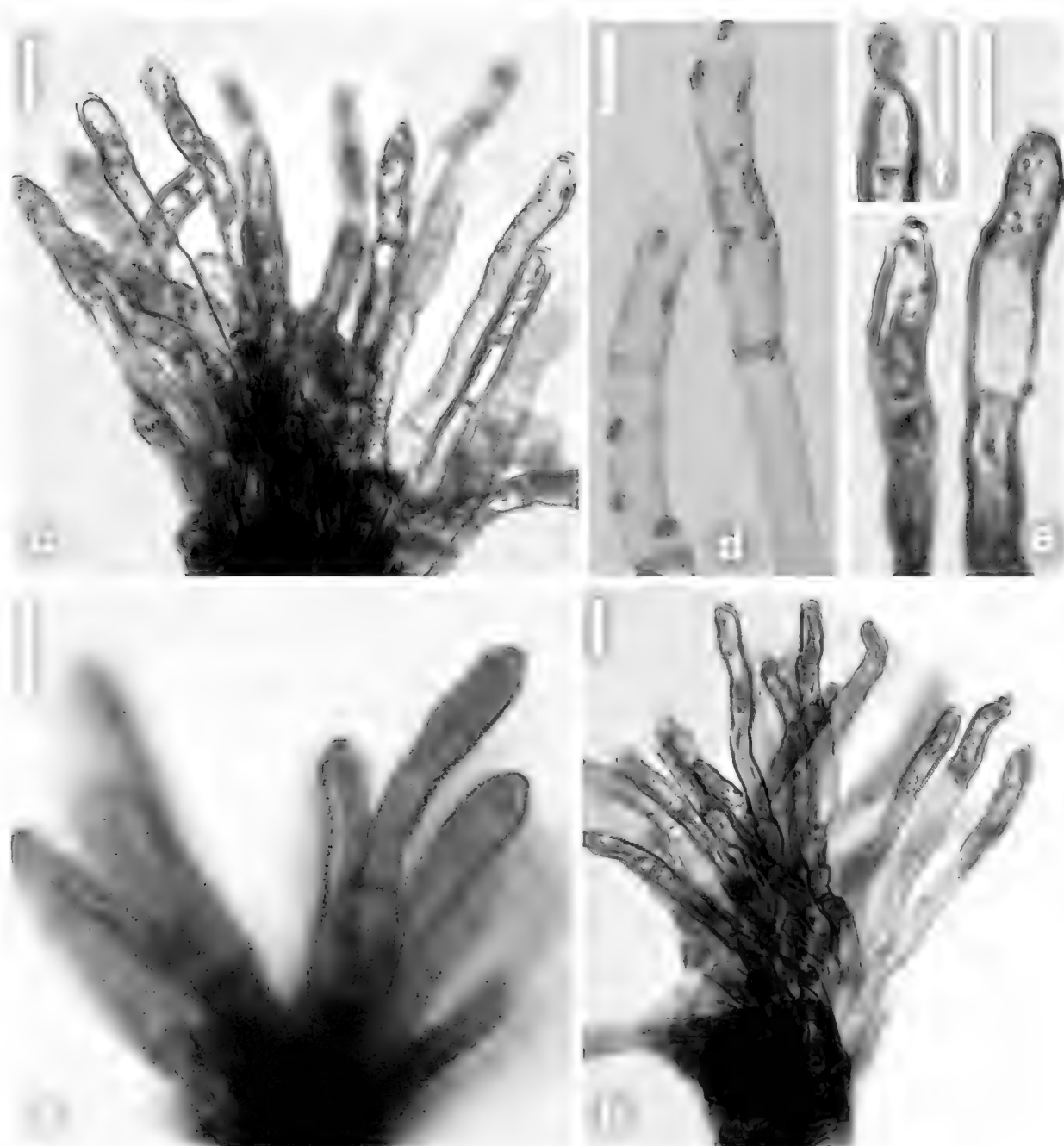
FIG. 2. *Distocercospora curvulata* (ex holotype, AMH 10239). a. Initial stage of development of conidiophores; b, c. Stromata with fasciculate conidiophores; d, e. Conidiogenous cells with protuberant loci; f. Conidiogenous cell with developing conidium. Scale bars = 10 μm.

COMMENTS— Three *Distocercospora* are currently accepted (www. indexfungorum.org; accessed 13 December 2020). Morphologically, the conidiophores of *D. indica* N.K. Verma & A.N. Rai and *D. pachyderma* (Syd. & P. Syd.) N. Pons & B. Sutton are much longer and more septate compared with *D. curvulata* (TABLE 1). The new species resembles *D. africana* Crous & U. Braun, which has shorter, thicker, branched, and less septate conidiophores and narrower conidia without any euseptation.

Fɪɢ. 3. Distocercospora curvulata (ex holotype, AMH 10239). Conidia. Scale bars = 10 μm.

TABLE 1. Comparative morphology of *Distocercospora* species.

Species.	Stromata (μm)	Conidiophores			Conidia		Host	Reference
		Septation	Nature	Size (μm)	Septation	Size (μm)		
D. africana	15–50 × 10–40	1–2(3)	B	15–80 × 3–10	1–5(6)-distoseptate	30–110 × 3–5	*Dioscorea* sp. (*Dioscoreaceae*)	Crous & Braun 1994
D. curvulata	12–46 × 8–30	1–7	UB	34–92 × 3.5–7	0–3-euseptate; 0–5-distoseptate	22–80 × 2–7	*Causonis trifolia* (*Vitaceae*)	This paper
D. indica	30–160	Pluriseptate	B	80–1000 × 4–6	0–5-distoseptate	25–90 × 4–5	*Holoptelea integrifolia* (*Ulmaceae*)	Verma & Rai 2014
D. pachyderma	Absent	Pluriseptate	B	316–490 × 3–5	1–5-distoseptate	46.5–94.5 × 4.5–6.5	*Dioscorea* spp. (*Dioscoreaceae*)	Kirschner & al. 2004

B = branched, UB = Unbranched.

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Apicheirospora sinensis gen. & sp. nov. from China

Jing-Wen Liu[1], Ya-Fen Hu[1], Kai Zhang[2], Xiu-Guo Zhang[3], Rafael F. Castañeda-Ruíz[4], Jian Ma[1]*

[1] *College of Agronomy, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, China*

[2] *College of Forestry Engineering, Shandong Agriculture and Engineering University, Jinan, Shandong 250100, China*

[3] *Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests, College of Plant Protection, Shandong Agricultural University, Taian, Shandong 271018, China*

[4] *Instituto de Investigaciones Fundamentales en Agricultura Tropical 'Alejandro de Humboldt' (INIFAT), Académico Titular de la "Academia de Ciencias de Cuba", Calle 1 Esq. 2, Santiago de Las Vegas, C. Habana, Cuba, C.P. 17200*

* Correspondence to: *majian821210@163.com; jxaumj@126.com*

Abstract—A new anamorphic genus and species, *Apicheirospora sinensis*, collected on dead branches in the Nabanhe Nature Reserve, Yunnan Province, China, is described and illustrated. The fungus is distinguished by macronematous, unbranched conidiophores with monoblastic, integrated, terminal, determinate or percurrently elongating conidiogenous cells, producing smooth conidia, which are holoblastic, solitary, acrogenous, dictyoseptate, variable in shape, cheiroid, applanate, occasionally branched, composed of several compact rows of cells, which form a compact cluster after successively dichotomous branching, brown to pale brown, evenly pigmented, with hyaline appendages.

Key words—asexual *Ascomycota*, hyphomycetes, new taxa, taxonomy

Introduction

The Nabanhe Nature Reserve is located in Xishuangbanna Prefecture, Yunnan Province, China, and in the western side of the Lancang River. It lies in 22.07–22.28°N 100.53–100.73°E and covers an area of 266 km². The average annual minimum and maximum temperatures are 18–22°C, and annual precipitation is 1100–1600 mm. The main forest types are tropical rainforest,

tropical monsoon forest and subtropical evergreen broad-leaved forest (Chao 2010). Such conditions create a very wide range of habitats favoring the growth of various microbial species. However, its mycobiota, especially microfungi, is poorly understood. During our mycological surveys in this reserve, an interesting fungus was collected from dead branches. Close examination found significant differences from previously described hyphomycetes (Seifert & al. 2011), and a new genus, *Apicheirospora*, is erected for it.

Materials & methods

Samples of dead branches were collected from humid environments and river bank in the forest ecosystems of the Nabanhe Nature Reserve, Yunnan Province, China, and placed in Ziploc™ bags for transporting to the laboratory, where the samples were processed and examined as described by Ma & al. (2011). Fungi were mounted in a drop of lactic acid on microscope slides, examined and photographed with an Olympus microscope (model BX 53), with a 100× (oil immersion) objective at the same background and scale. Adobe Photoshop 7.0 was used for image processing to assemble photomicrographs into a plate. Isolation of the fungus failed on potato dextrose agar (PDA) at 25°C after several attempts. The specimen was deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, Jiangxi, China (HJAUP).

Taxonomy

Apicheirospora Jing W. Liu, X.G. Zhang, R.F. Castañeda & Jian Ma, **gen. nov.**
IF 559613

> Differs from *Digitoramispora* by its applanate, cheiroid, evenly pigmented conidia with appendages and several compact rows of cells; from *Xiuguozhangia* by its integrated, terminal conidiogenous cells; and from *Piricaudiopsis* by its monoblastic conidiogenesis.

> Type species: *Apicheirospora sinensis* Jing W. Liu & al.

> Etymology: *api-* (Latin) meaning apiculate + *cheirospora* (Latin) referring to the type of conidia of this anamorphic fungus.

Conidiophores macronematous, mononematous, unbranched, erect, smooth, septate, brown to dark brown. Conidiogenous cells monoblastic, integrated, terminal, determinate or indeterminate, with several percurrent elongations, dark brown to brown. Conidial secession schizolytic. Conidia solitary, acrogenous, eudictyoseptate, cheiroid, applanate, composed of several compact, subparallel rows of cells, produced after successively dichotomous

branching, brown to pale brown, sometimes the apical cells produce unbranched or branched, septate, pale brown to subhyaline appendages.

Apicheirospora sinensis Jing W. Liu, X.G. Zhang, R.F. Castañeda & Jian Ma, **sp. nov.**   Fig. 1

IF 559614

Differs from *Digitoramispora* spp. by its applanate, cheiroid, evenly pigmented conidia with appendages and several subparallel, compact rows of cells.

Type: Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, the Nabanhe Nature Reserve, on dead branches of an unidentified broadleaf tree, 12 July 2021, J.W. Liu (**Holotype**, HJAUP M2074).

Etymology: refers to China, in which the fungus was collected.
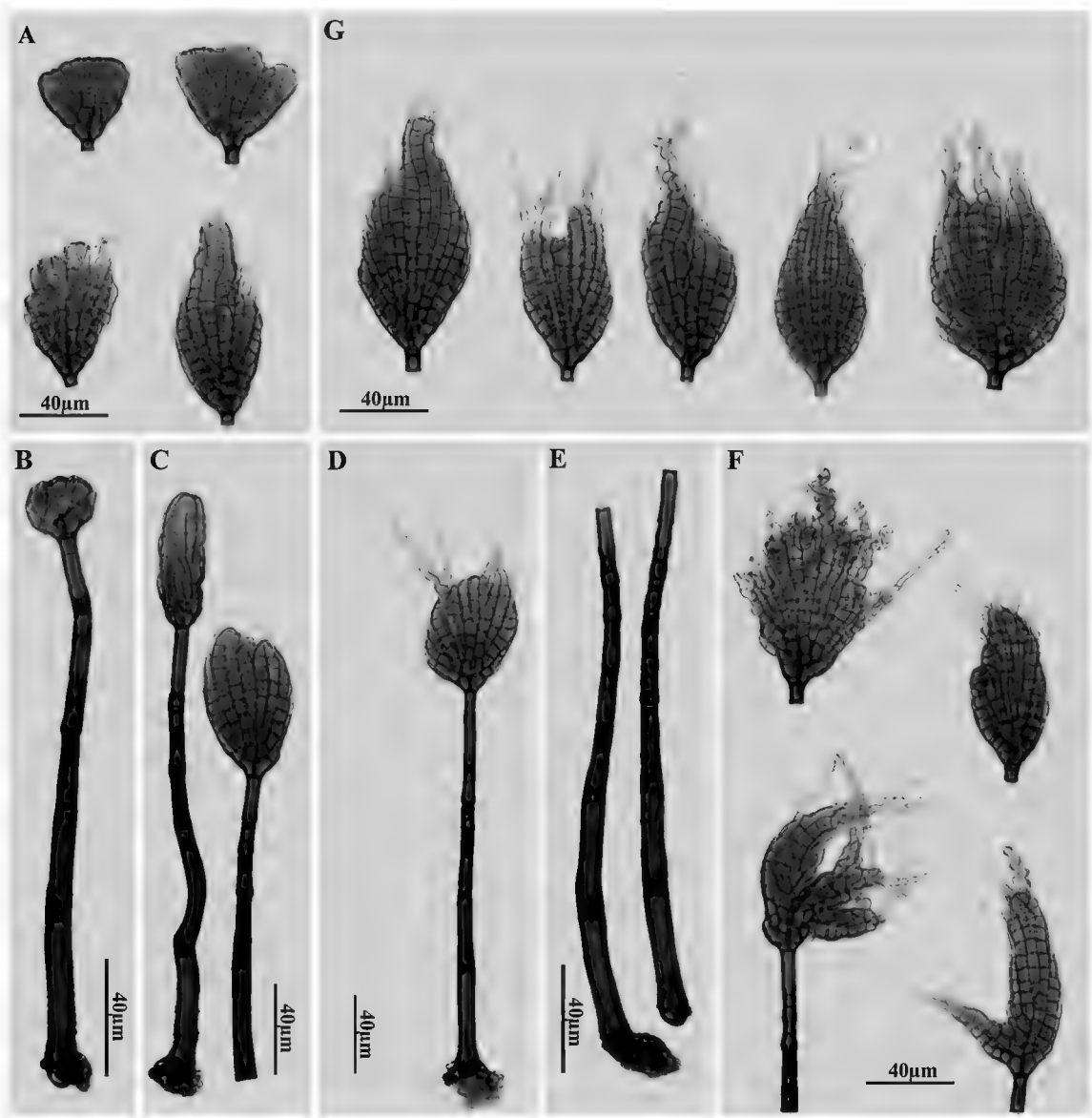
Fig. 1. *Apicheirospora sinensis* (holotype, HJAUP M2074). A. Developing conidia; B–D. Conidiophores, conidiogenous cells, and conidia; E. Conidiophores and conidiogenous cells; F. Conidiogenous cells and conidia. G. Conidia.

COLONIES on the natural substrate effuse, dark brown, hairy. Mycelium partly superficial and immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae. CONIDIOPHORES macronematous, mononematous, single, unbranched, erect, straight or slightly flexuous, cylindrical, smooth, 5–14-septate, thick-walled, dark brown to brown, 125–215 × 7.5–10 µm. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, determinate or with several percurrent elongations, cylindrical, truncate at the apex following conidium disarticulation, smooth, dark brown to brown, 13–20 × 5–6.5 µm. Conidial secession schizolytic. CONIDIA solitary, dry, acrogenous, eudictyoseptate, variable in shape, cheiroid, applanate, composed of several compact, subparallel rows of cells, mostly forming 1(–2) compact cluster produced after successive dichotomous branching, brown to pale brown, smooth, 67.5–113 × 32.5–60 µm; cuneate basal cell, sometimes the apical cells producing slender, smooth, simple or branched, divergent, straight, curved to coiled, septate, pale brown to subhyaline appendages, up to 80 µm long, 2.5–4 µm wide.

Discussion

Apicheirospora sinensis is unique in its monoblastic, integrated, terminal, determinate or percurrently elongating conidiogenous cells on macronematous, unbranched conidiophores. It develops smooth conidia that are holoblastic, solitary, acrogenous, dictyoseptate, applanate, cheiroid, occasional branched, composed of several compact rows of cells, that form a compact cluster after successive dichotomous branching, brown to pale brown, evenly pigmented, with appendages and schizolytic conidial secession. It has the same conidial ontogeny as *Ceratosporella* Höhn. (Höhnel 1923), *Digitoramispora* R.F. Castañeda & W.B. Kendr. (Castañeda-Ruíz & Kendrick 1990), *Pseudoacrodictys* W.A. Baker & Morgan-Jones (Baker & Morgan-Jones 2003), and *Xiuguozhangia* K. Zhang & al. (Zhang & al. 2014). However, *Digitoramispora* differs by its dictyoseptate conidia with subhyaline or colorless terminal cell of each row; *Pseudoacrodictys* by its subglobose, broadly pyriform to turbinate or somewhat irregularly shaped conidia bearing numerous septa arranged in an oblique fashion with internal cells often becoming compressed and contorted; *Ceratosporella* by its divergent and arm-shaped conidia with all arms arising from the basal cell; and *Xiuguozhangia* by its discrete conidiogenous cells with conidia bearing 0–4 appendages. *Apicheirospora* is also compared with *Piricaudiopsis* J. Mena & Mercado (Mena-Portales & Mercado-Sierra 1987) because of their similar conidial morphology, but *Piricaudiopsis* has

monotretic, integrated and discrete conidiogenous cells, which differ from the monoblastic conidiogenesis of *Apicheirospora*. *Distoceratosporella* J.S. Monteiro & al. and *Pararhexoacrodictys* Cantillo & Gusmão are somewhat similar to *Apicheirospora*, but *Distoceratosporella* has distoseptate conidial arms that arising from the basal cell (Monteiro & al. 2017); and *Pararhexoacrodictys* conidia have thin septa than appear to be collapsed or distorted, with basal cell delimited by a transversal septum, and seceding rhexolytically (Cantillo & al. 2019).

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Porosynnema catenatum gen. & sp. nov. from Yunnan, China

Jing-Wen Liu[1], Ya-Fen Hu[1], Xiu-Guo Zhang[2], Rafael F. Castañeda-Ruíz[3], Jian Ma[1]*

[1] *College of Agronomy, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, China*
[2] *Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests, College of Plant Protection, Shandong Agricultural University, Taian, Shandong 271018, China*
[3] *Instituto de Investigaciones Fundamentales en Agricultura Tropical 'Alejandro de Humboldt' (INIFAT), Académico Titular de la "Academia de Ciencias de Cuba", Calle 1 Esq. 2, Santiago de Las Vegas, C. Habana, Cuba, C.P. 17200*

* Correspondence to: *majian821210@163.com; jxaumj@126.com*

Abstract—A new genus and species, *Porosynnema catenatum*, collected on decaying twigs of an unidentified broadleaf tree in Xishuangbanna, China, are described and illustrated. The genus is characterized by macronematous, unbranched conidiophores forming distinct, indeterminate synnematal conidiomata with polytretic, integrated, terminal, cylindrical, cicatrized, determinate or percurrently extending conidiogenous cells that produce acropleurogenous, catenate, obclavate, distoseptate conidia with a thick, dark hilum at the base. A key to *Porosynnema* and its morphologically similar genera is provided.

Key words—asexual *Ascomycota*, taxonomy, tropical forest

Introduction

Xishuangbanna Dai Autonomous Prefecture is located in the southwestern part of Yunnan Province, lying on the northern edge of the tropical region south of the Tropic of Cancer. It covers 19,125 km² and has a tropical monsoon climate with high humidity, high mean temperature, and an annual precipitation of 1200–1800 mm, so it is particularly suitable for the growth of microscopic fungi in plant remains. During our continuous survey of anamorphic fungi in this region, an interesting fungus was collected that is significantly different

from all previously described hyphomycetes (Seifert & al. 2011). Therefore, it is described here as a new genus and species.

Materials & methods

Samples of dead branches were collected from humid environments and river bank in the forest ecosystems of Xishuangbanna, Yunnan Province, China, and placed in Ziploc™ bags for transport to the laboratory, where they were processed, and examined as described by Ma & al. (2011). Fungi were mounted in a drop of lactic acid on microscope slides and examined and photographed using an Olympus microscope (model BX 53) with a 100× (oil immersion) objective at the same background and scale. Adobe Photoshop 7.0 was used for image processing to assemble photographic plates. Single-spore isolations were unsuccessful on potato dextrose agar (PDA) after several attempts. The specimens are deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, Jiangxi, China (HJAUP).

Taxonomy

Porosynnema Jing W. Liu, X.G. Zhang, R.F. Castañeda & Jian Ma, **gen. nov.**
IF 559619

Differs from *Dendrographium* by its cicatrized conidiogenous cells, and catenate conidia.

Type species: *Porosynnema catenatum* Jing W. Liu & al.

Etymology: *poro-* (Latin) meaning pore + *-synnema* (Latin) referring to the type of conidiomata of this fungus.

Conidiomata synnematous, solitary, erect, cylindrical, indeterminate, melanocratic. Conidiophores synnematous, macronematous, unbranched, septate, melanocratic. Conidiogenous cells diverging from the synnematal axis, polytretic, terminal, integrated, cylindrical, cicatrized, determinate or with percurrent extensions. Conidial secession schizolytic. Conidia acropleurogenous, blastocatenate, obclavate, distoseptate, generally with a thick, dark hilum at the base.

Porosynnema catenatum Jing W. Liu, X.G. Zhang, R.F. Castañeda & Jian Ma,
sp. nov. Figs 1, 2
IF 559620

Differs from *Dendrographium* spp. by its cicatrized conidiogenous cells, and catenate conidia.

Type: China, Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, the Nabanhe National Nature Reserve, on dead branches of an unidentified broadleaf tree,

12 July 2021, J.W. Liu (**Holotype**, HJAUP M2152).

Etymology: Latin, *catenatum*, referring to the chained conidia.
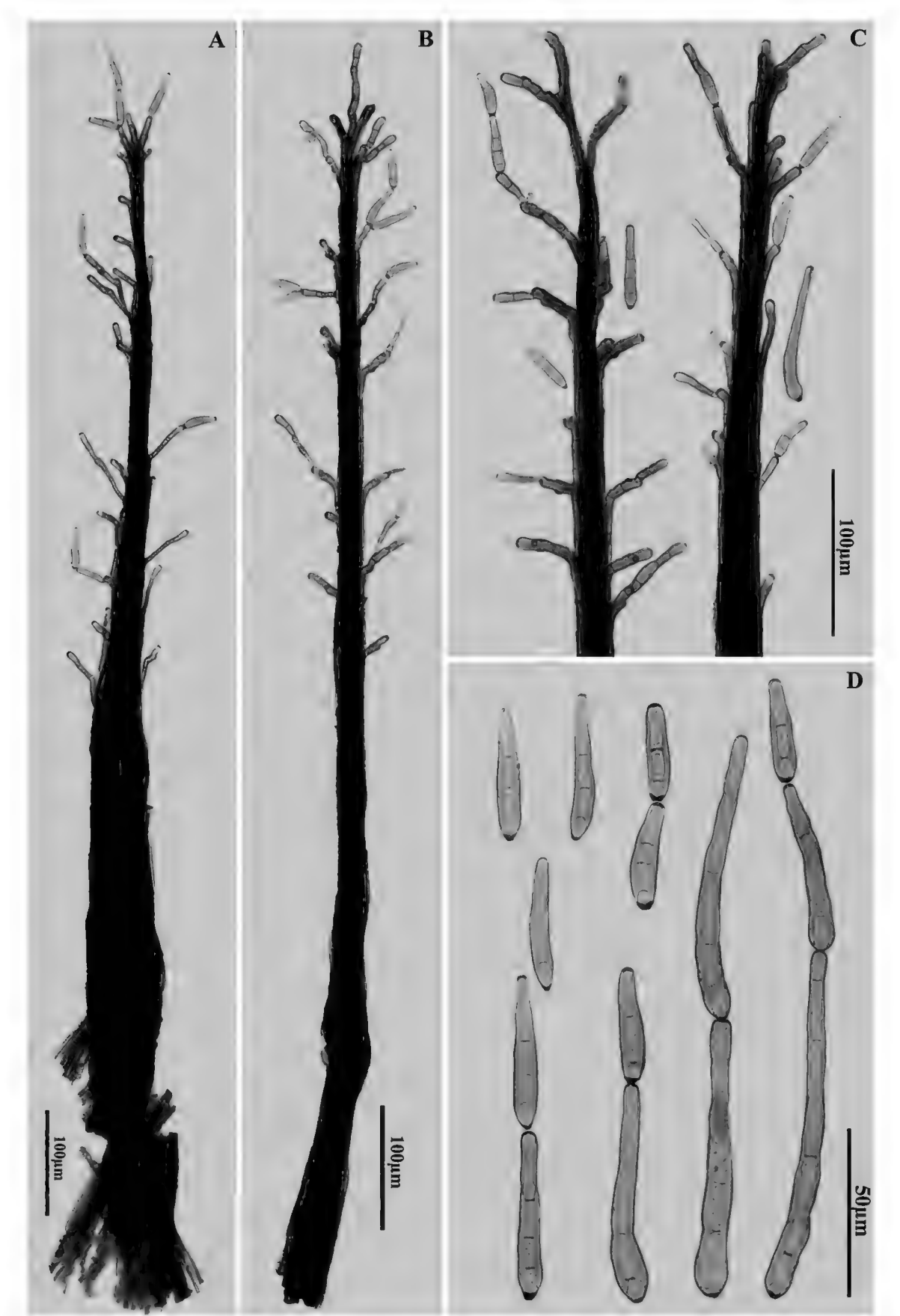
Fig. 1. *Porosynnema catenatum* (holotype, HJAUP M2152). A–C. Synnemata with conidiophores, conidiogenous cells, and conidia; D. Conidia.
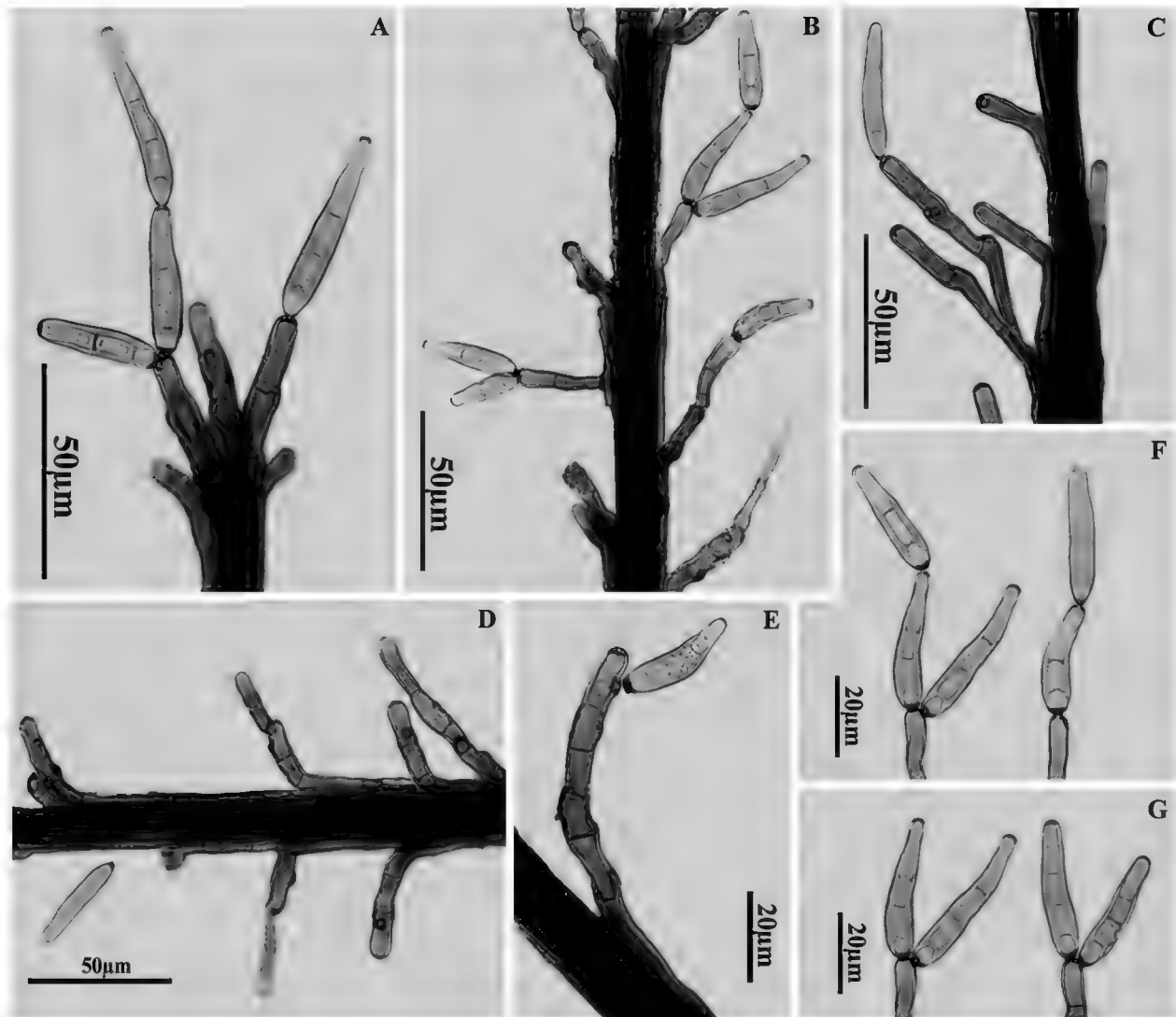
Fig. 2. *Porosynnema catenatum* (holotype, HJAUP M2152). A–E. Conidiophore section, conidiogenous cells, and conidia; F, G. Conidiogenous cells, and conidia.

Colonies effuse on the natural substratum, dark brown to black, hairy. Mycelium superficial and immersed. Conidiomata synnematous, solitary, erect, cylindrical, dark brown to black, indeterminate, up to 1.9 mm high, 21–54 μm diam., with lateral and terminal divergent conidiophores. Conidiophores macronematous, unbranched, with filaments cylindrical, compactly packed, slightly bent at the apex, laterally and apically divergent on the stipe, septate, dark brown to brown, smooth-walled, up to 1.9 mm long, 3–6 μm wide. Conidiogenous cells polytretic, integrated, terminal, cylindrical, cicatrized, smooth, brown to pale brown, determinate or with 1–2 cylindrical percurrent extensions, 14–29 × 4–6 μm. Conidial secession schizolytic. Conidia acropleurogenous, blastocatenate, obclavate, straight or slightly curved, 2–5-distoseptate, smooth-walled, grey-brown, paler toward the

apex, 28–94 × 6–8 μm, tapered towards the apex, base truncate, 2–4 μm wide, with a thick, dark hilum at the base.

Discussion

Porosynnema is distinguished by its unbranched conidiophores forming synnematous conidiomata, and solitary, acropleurogenous, catenate, obclavate, distoseptate conidia seceding schizolytically from polytretic, terminal, integrated, determinate or percurrently extending conidiogenous cells diverging from the axis of the synnemata. It appears like several genera including *Podosporium* Schwein., *Dendrographium* Massee, *Vamsapriya* Gawas & Bhat, and *Podosporiopsis* Jian Ma & al. in possessing distinct conidiophores forming synnematous conidiomata (Schweinitz 1832, Massee 1892, Gawas & Bhat 2005, Ma & al. 2016). However, in *Vamsapriya*, *Podosporium* and *Podosporiopsis* conidiogenesis is monotretic, in contrast to the polytretic conidiogenesis of *Porosynnema*. In addition, *Vamsapriya* and *Podosporium* have euseptate conidia far different from the distoseptate conidia of *Porosynnema*. The conidiogenous events in *Dendrographium* and *Porosynnema* are the same, but in *Dendrographium* the synnemata are determinate and the conidia are formed solitarily on the conidiogenous loci (Ellis 1971), while *Porosynnema* has indeterminate synnemata and the conidia are blastocatenate formed on the conidiogenous loci.

Several other genera, including *Exosporium* Link, *Corynespora* Güssow, *Neopodoconis* Rifai and *Ellismarsporium* R. F. Castañeda & X.G. Zhang are also compared with *Porosynnema* (Link 1809, Güssow 1905, Rifai 2008, Castañeda-Ruíz & al. 2017). These genera have tretic conidiogenesis and similar conidial morphology, but *Porosynnema* has a synnematous conidiomata, which is not found in *Exosporium*, *Corynespora* and *Ellismarsporium*. In addition, *Corynespora* differs by its monotretic conidiogenous cells, and *Exosporium* and *Neopodoconis* differ by their conidiogenous cells with dark and prominent scar at the conidiogenous loci (Ellis 1971, Rifai 2008). *Corynesporella* Munjal & H.S. Gill, characterized by mononematous, branched conidiophores, and monotretic conidiogenous cells which produce solitary or blastocatenate, distoseptate conidia, is superficially similar to *Porosynnema* (Munjal & Gill 1961). *Porosynnema* shares some characters with *Poroisariopsis* M. Morelet, such as synnematous conidiomata, polytretic conidiogenous cells, and distoseptate conidia, but in *Poroisariopsis* the conidia are solitary (Morelet 1971, Heredia & al. 2020).

Key to *Porosynnema* and morphologically similar genera

1. Conidiophores mononematous 2
1. Conidiophores synnematous 6
2. Conidiogenous cells monotretic 3
2. Conidiogenous cells polytretic 4
3. Conidiophores unbranched .... *Corynespora*
3. Conidiophores branched .... *Corynesporella*
4. Conidia euseptate .... *Neopodoconis*
4. Conidia distoseptate 5
5. Conidiogenous cells cicatrized, with dark and prominent scars; conidia usually solitary, shortly catenate in one species .... *Exosporium*
5. Conidiogenous cells non-cicatrized; conidia blastocatenate .... *Ellismarsporium*
6. Conidiogenous cells monotretic 7
6. Conidiogenous cells polytretic 9
7. Conidia distoseptate .... *Podosporiopsis*
7. Conidia euseptate 8
8. Conidia catenate .... *Vamsapriya*
8. Conidia solitary .... *Podosporium*
9. Conidia catenate, distoseptate, conidiogenous cells cicatrized .... *Porosynnema*
9. Conidia solitary 10
10. Synnemata indeterminate, conidiogenous cells with cylindrical, uncinate to sigmoid, enteroblastic, percurrent extensions .... *Poroisariopsis*
10. Synnemata determinate, conidiogenous cells not as above .... *Dendrographium*

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MYCOTAXON

Four species of *Rhizocarpon* subg. *Phaeothallus* in China

Yan-xue Bi[1], Yan-meng Zhang[1], Zun-tian Zhao[2*], Ling Hu[1*]

[1] *Institute of Environment and Ecology, Shandong Normal University,*
   Jinan, 250014, P. R. China
[2] *Key Laboratory of Plant Stress Research, College of Life Sciences,*
   Shandong Normal University, Jinan, 250014, P. R. China
Correspondence to: Hu_Ling_123@163.com, ztzhao@sdnu.edu.cn

Abstract—Three lichen species are reported from China for the first time: *Rhizocarpon cinereovirens*, *R. rittokense*, and *R. roridulum*. A revision of the Chinese material determined as *R. infernulum* f. *infernulum* shows that it belongs to *R. infernulum* f. *sylvaticum*. The morphology, secondary chemistry, ecology, and distribution ranges of the species are investigated and discussed. An identification key is given to the species of *Rhizocarpon* subg. *Phaeothallus* in China.

Key words—*Rhizocarpaceae, Rhizocarpales*, saxicolous, taxonomy

Introduction

Rhizocarpon (*Rhizocarpaceae*) was validated by De Candolle in 1805 (Kirk & al. 2008). It is widely distributed on rocks or occurs as parasite on other lichens throughout the world, especially in alpine and polar regions (Matwiejuk 2008). *Rhizocarpon* is characterized by having crustose thallus, usually distinct black prothallus, lecideine apothecia, richly branched and anastomosing paraphyses, *Rhizocarpon*-type and (1–)8-spored asci and hyaline to brown or olive-green, halonate, ellipsoid ascospores that can be transversely septate or submuriform to densely muriform (Feuerer & Timdal 2004, Fletcher & al. 2009, McCarthy & Elix 2014). Thomson (1967) divided the genus *Rhizocarpon* into taxa with a yellow thallus containing rhizocarpic acid (*R.* subg. *Rhizocarpon*) and taxa with

non-yellow (white, ashy, or brown) thallus lacking rhizocarpic acid (*R.* subg. *Phaeothallus*).

Rhizocarpon includes about 235 species worldwide (Lücking & al. 2016; Davydov & Yakovchenko 2017; Etayo 2017; Kalb & Aptroot 2017; Paukov & al. 2017; Kondratyuk & al. 2018; Fryday 2019; Elix & McCarthy 2019; McCarthy & al. 2020; Spribille & al. 2020), of which 47 have been reported from China (Wei 1991, 2020; Abbas & Wu 1998; Aptroot 2002; Aptroot & Sparrius 2003; Sérusiaux & al. 2003; Golubkov & Maywiejuk 2009; Li & al. 2013; Zhao & al. 2013; Mahire & al. 2015; Wang & al. 2015a,b,c, 2016; Gulina & Anwar 2019; Hu & al. 2020).

During our study of *Rhizocarpon* in China, three additional species have been found: *R. cinereovirens, R. rittokense,* and *R. roridulum*; a revision of the Chinese material determined as *R. infernulum* f. *infernulum* showed that it belongs to *R. infernulum* f. *sylvaticum.* An identification key is provided for the 31 species of *Rhizocarpon* subg. *Phaeothallus* known from China.

Materials & methods

The examined specimens are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU) and the Sectio Lichenum of Herbarium Mycologici Academiae Sinicae, Chinese Academy of Sciences, Beijing, China (HMAS-L). Macromorphological characters were examined under a stereomicroscope (COIC XTL7045B2) and micromorphological characters under a polarizing microscope (Olympus CX41). For identification, the thallus and medulla were tested with K (a 10% aqueous solution of potassium hydroxide), C (a solution of aqueous sodium hypochlorite), I (Lugol's iodine), N (a 50% aqueous solution of nitric acid), and P (a saturated solution of p-phenylenediamine in 95% ethyl alcohol). Lichen substances were identified using standardized thin-layer chromatography techniques (TLC) with solvent system C (Orange & al. 2010). Photographs were taken with an Olympus SZX16 and a BX61 microscope with a DP72 lens.

Taxonomy

Rhizocarpon cinereovirens (Müll. Arg.) Vain., Acta Soc. Fauna Fl. Fenn. 53(1): 336 (1922)   Fig. 1

THALLUS crustose, cream-colored to pale brown, areolate to bullate, 2–5 cm diam.; areoles rounded to angular, flat to convex, smooth, continuous, 0.2–0.4 mm diam. PROTHALLUS absent. MEDULLA I–. APOTHECIA lecideine, black, sessile to somewhat immersed, 0.3–0.6 mm diam., rounded to angular,
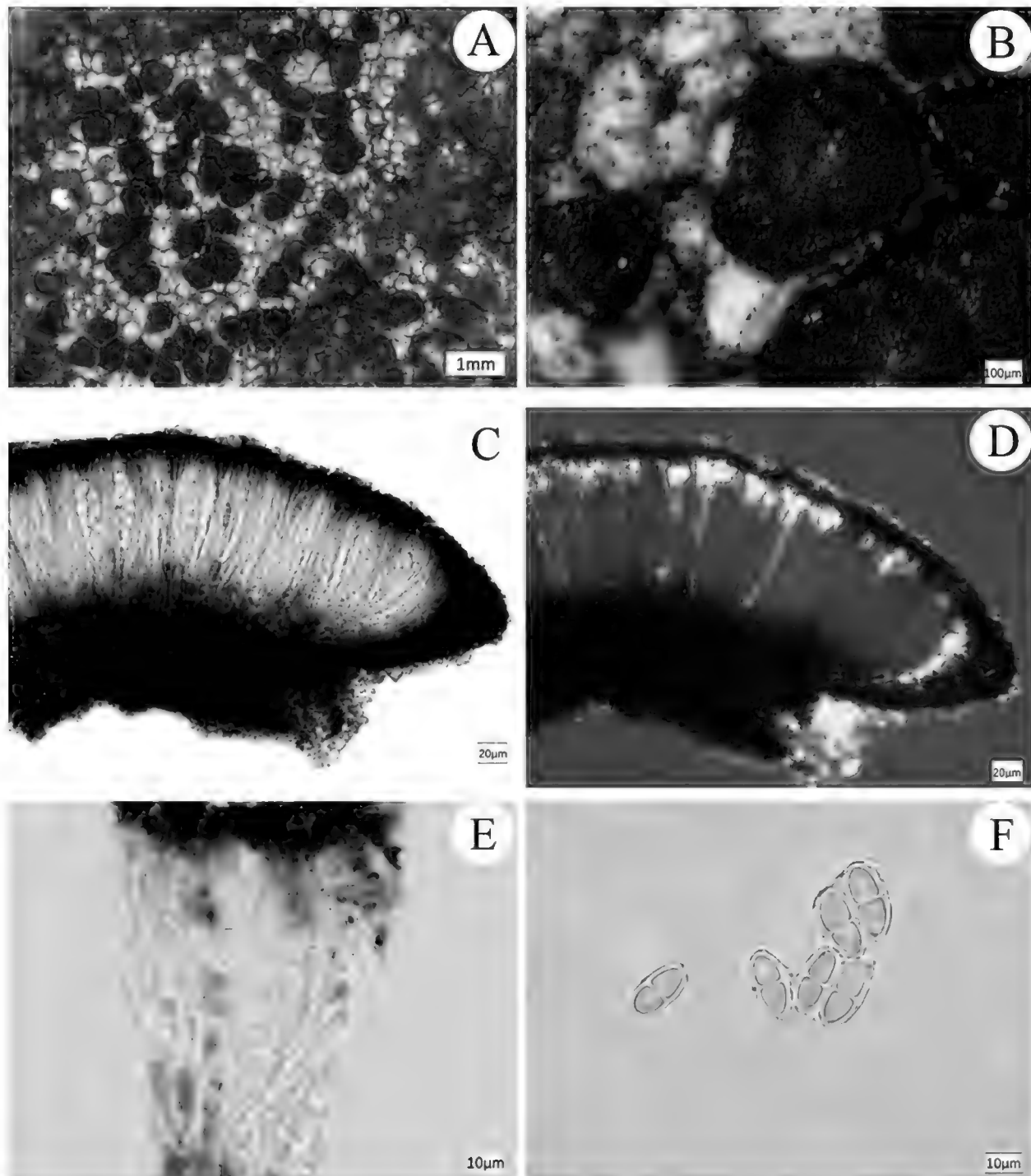
Fig. 1. *Rhizocarpon cinereovirens* (HMAS-L–Gao114944). A. Thallus; B. Apothecia; C. Apothecium section; D. Crystals in exciple and epihymenium; E. Ascus; F. Ascospores. Scale bars: A = 1 mm; B = 100 µm; C, D = 20 µm; E, F = 10 µm.

flat to weakly convex; margin thin, epruinose. Exciple dark brown, K–, with crystals dissolving in K; epihymenium dark green, K+ green, with crystals dissolving in K; hymenium hyaline; paraphyses branched and anastomosing, scarcely swelling at apex, with a green-blue cap; hypothecium dark brown. Asci 8-spored. Ascospores hyaline, 1-septate, 15–17 × 6.5–8.5 µm, halonate. Conidiomata not seen.

CHEMISTRY—Medulla K+ yellow turning red, C–, P+ yellow. Norstictic acid and stictic acid detected by TLC.

SPECIMEN EXAMINED: **CHINA. JILIN, Shulan City**, Qingsong forestry station, alt. 600 m, on siliceous rock, 8 Oct. 1984, Xiangqun Gao 114944 (HMAS-L).

DISTRIBUTION—Norway, Denmark, Great Britain, Finland, Sweden, Switzerland, Canada, USA (Thomson 1967, Fryday 2002). New to China and to Asia.

COMMENTS—Our specimen conforms to previously published descriptions (Fryday 2002, Fletcher & al. 2009). *Rhizocarpon cinereovirens* is characterized by the cream-colored to pale brown thallus, dark green epihymenium with crystals, hyaline, 1-septate ascospores and by containing norstictic and stictic acid. *Rhizocarpon cinereovirens* is somewhat similar to *R. discoense* Lynge by paraphyses with greenish cap at apex and hyaline 1-septate ascospores, but *R. discoense* has a well-developed exciple with a violet pigmentation (K+ violet), a thallus with grey bullate areoles, and wider ascospores 16–17 × 9–10 μm (Fryday 2002, Fletcher & al. 2009). *Rhizocarpon cinereovirens* is also similar to *R. infernulum* (Nyl.) Lynge f. *infernulum*, which has a more well-developed exciple with a hyaline interior, and less richly branched and anastomosing paraphyses with more sharply defined caps that are brown rather than green. In additon, the lichen substances of *R. infernulum* f. *infernulum* are ± stictic acid (Fryday 2002, Fletcher & al. 2009).

Rhizocarpon rittokense (Hellb.) Th. Fr., Lichenogr. Scand. 2: 615 (1874)　　FIG. 2

THALLUS crustose, brown, areolate to subsquamulose, 3–4 cm diam.; areoles peltate, concave, shining, smooth, scattered, often with pruinose margin, 0.2–1 mm diam. PROTHALLUS present, black. MEDULLA I–. APOTHECIA lecideine, black, immersed, 0.2–0.5 mm diam., rounded, flat; margin thin, epruinose. EXCIPLE dark brown, K+ violet, with crystals dissolved in K; epihymenium brown, K+ violet, with crystals dissolved in K; hymenium hyaline; paraphyses branched and anastomosing, with a sharply delimited, brown-pigmented cap; hypothecium dark brown. ASCI 8-spored. ASCOSPORES brown-green to dark brown, 1-septate, 18–23 × 11.5–15 μm, halonate. CONIDIOMATA not seen.

CHEMISTRY—Medulla K–, C–, P–. Barbatic acid detected by TLC.

SPECIMENS EXAMINED: **CHINA. SICHUAN, Luding Co.**, Moxi Town, Yajiagen, alt. 4000 m, 29.9100°N 102.0038°E, on siliceous rock, 13 Oct. 2015, Weicheng Wang, Xiangxiang Zhao, Feixiang Shi, Zuntian Zhao 20150587 (SDNU). **YUNNAN, Lijiang City**, Mt. Laojun, alt. 3850 m, on siliceous rock, 25 Aug. 2015, Weicheng Wang 20150513 (SDNU).

Fig. 2. *Rhizocarpon rittokense* (SDNU–Wang20150587). A. Thallus and apothecia; B. Apothecium section; C. Crystals in exciple and epihymenium; D. K reaction; E. Paraphyses; F. Ascus; G. Ascospores. Scale bars: A = 200 μm; B–D = 20 μm; E–G = 10 μm.

DISTRIBUTION—Sweden, Denmark, Norway, Iceland, Canada, USA, Russia, Norway (Lynge 1932, Thomson 1967, Timdal & Holtan-Hartwig 1988). New to China.

COMMENTS—Our specimens conform to previously published descriptions, except for the presence of the brown-green ascospores (Thomson 1967).

Rhizocarpon rittokense is characterized by K+ violet exciple with crystals, K+ violet epihymenium with crystals and the subsquamulose thallus. *Rhizocarpon rittokense* is similar in habitat and 1-septate ascospores to *R. groenlandicum* Lynge, which differs by its convex apothecia with a bluish hymenium and its smaller ascospores (17–21 × 10–11 μm; Lynge 1932). *Rhizocarpon rittokense* is also similar to *R. cinereonigrum* Vain. in K+ violet epihymenium and exciple with crystals, but *R. cinereonigrum* differs from *R. rittokense* by K– exciple and larger ascospores (28–38 × 12–l8 μm; Hu & al. 2020).

Rhizocarpon roridulum (Th. Fr.) Malme, Svensk Bot. Tidskr. 8(3): 279, 291 (1914)   Fig. 3

Thallus crustose, gray to grayish brown, rimose to areolate, 1.5–2 cm diam.; areoles angular, flat, matt, scattered, 0.2–0.5 mm diam. Prothallus absent. Medulla I–. Apothecia lecideine, black, 0.3–0.5 mm diam., somewhat immersed, rounded to angular, weakly concave; margin thick, epruinose. Exciple dark brown, K+ violet, without crystals; epihymenium brown, K+ violet, without crystals; hymenium hyaline; paraphyses branched and anastomosing, scarcely swelling at apex, without strongly delimited pigmented cap; hypothecium dark brown. Asci 8-spored. Ascospores hyaline, submuriform, with 5–9 cells in optical section, 23–30 × 10.5–15 μm, halonate. Conidiomata not seen.

Chemistry—Medulla K–, C–, P–. No substance detected by TLC.

Specimen examined: CHINA. Sichuan, Litang Co., Mt. Qiazila, alt. 4250 m, on siliceous rock, 16 Oct. 2015, Zuntian Zhao, Feixiang Shi, Xiangxiang Zhao, Weicheng Wang 20150697 (SDNU).

Distribution—Finland, Sweden, Norway (Fries 1874, Ihlen 2004). New to China and to Asia.

Comments—Our specimen conforms to the description of Fries (1874). *Rhizocarpon roridulum* is characterized by the brown to grey-brown thallus, pruinose apothecia (but sometimes the pruina is missing), K+ violet epihymenium, and by not containing lichen substances (Ihlen 2004, Hu & al. 2020). *Rhizocarpon roridulum* is similar to *R. distinctum* Th. Fr. in the dark brown exciple and brown epihymenium, but *R. distinctum* usually has an I+ blue medulla, brown ascospores and contains stictic acid (Fryday 2000). *Rhizocarpon roridulum* is also similar in the K+ violet exciple to *R. geminatum* Körb., which can be distinguished by the 2-spored asci and larger ascospores (36–50 × 17–25 μm; McCarthy & Elix 2014). Additionally, *Rhizocarpon*

Fɪɢ. 3. *Rhizocarpon roridulum* (SDNU–Zhao20150697). A. Thallus; B. Apothecia; C. Apothecium section; D. K reaction; E. Paraphyses; F. Ascus; G. Ascospores. Scale bars: A = 500 μm; B = 400 μm; C, D = 50 μm; E, G = 10 μm; F = 20 μm.

roridulum is similar to *R. amphibium* (Fr.) Körb. in the predominantly reddish brown epihymenium with K+ purple reaction, but *R. amphibium* is characterized by the apothecia never with a white pruina and the exciple in section frequently with a green pigment in the outer and upper part (Ihlen 2004).

Revision

Rhizocarpon infernulum f. *sylvaticum* Fryday, Lichenologist 34(6): 468 (2002)

THALLUS crustose, brown, rimose to areolate, areoles rounded to angular, flat to weakly convex, matt, contiguous, 0.5 mm diam. PROTHALLUS absent. MEDULLA I–. APOTHECIA lecideine, black, 1 mm diam., sessile to somewhat immersed, rounded to angular, flat to weakly convex; margin thin, persistent, epruinose. EXCIPLE dark brown at the rim, inner part paler brown, K–, without crystals; epihymenium macrocarpa-green, K–, N+ red, without crystals; hymenium hyaline; paraphyses branched and anastomosing, with a sharply delimited, brown-pigmented cap; hypothecium dark brown. ASCI 8-spored. ASCOSPORES hyaline, 1-septate, (16–)17.5–20(–24) × 8–10 μm, halonate. CONIDIOMATA not seen.

CHEMISTRY—Medulla K–, C–, P–. No substance detected by TLC.

SPECIMENS EXAMINED: **CHINA. GUIZHOU, Leishan county,** Mt. Leigong, alt. 1800 m, on siliceous rock, 1 Apr. 2011, Yuliang Cheng 20112591A (SDNU); alt. 2100 m, on rock, 1 Apr. 2011, Xingran Kou 20111813 (SDNU); alt. 2700 m, on siliceous rock, 9 Nov. 2009, Lulu Zhang 20103078 (SDNU).

DISTRIBUTION—Great Britain, Ireland, Sweden, Canada, USA, China (Fryday 2002, Fletcher & al. 2009, Zhao & al. 2013).

COMMENTS—*Rhizocarpon infernulum* f. *sylvaticum* is characterized by the slightly larger spores and a smooth, cracked thallus, compared to the more areolate thallus and smaller spores (15–18 × 7–8.5 μm) of *R. infernulum* f. *infernulum*. Another difference between these two forms is the pigment of epihymenium: Fryday (2002) included the specimens with aeruginose (macrocarpa-green; K–, N+ red) to blue-black epihymenium in f. *infernulum*, and the specimens with invariably hyaline epihymenium in f. *sylvaticum*.

On the contrary, Möller (2021) found that the degree of epihymenium pigments varied within *R. infernulum* f. *sylvaticum*, some specimens had a very thin green epihymenium, and others had a very thick bright green epihymenium. During his investigation of the *R. hochstetteri* group by molecular methods, he found that specimens from clade A2 which he regarded as f. *sylvaticum* form a highly supported clade together with a specimen (11041) that Fryday himself determined as f. *sylvaticum*, and these specimens fit his observations of the type specimen. Moreover, the clade A2 (including f. *sylvaticum*) and A3 (including f. *infernulum*) were well supported and separated. He suggested (Möller 2021)

that f. *sylvatica* should be raised to species rank, but did not publish a formal proposal. Here, we retain the name *R. infernulum* f. *sylvaticum*.

Rhizocarpon infernulum f. *infernulum* was first reported from China by Zhao & al. (2013). Recently we re-examined those specimens (SDNU-20103078, 20111813, 20112591A) and we found that the spores measured (16–)17.5–20(–24) × 8–10 μm, much larger than the 15–18 × 7–8.5 μm published by Zhao & al. (2013). The specimen also had a thin, smooth, brown, cracked thallus, sessile apothecia with a persistent proper margin, so we reidentify the specimen as *R. infernulum* f. *sylvaticum*.

Key to the species of *Rhizocarpon* subg. *Phaeothallus* in China

1. Ascospores l-septate 2
1. Ascospores 3-septate to muriform 13
2. Ascospores remaining hyaline or only finally darkening 3
2. Ascospores soon becoming dark green or brown 7
3. Thallus dark brown, medulla I+ blue .... *R. polycarpum*
3. Thallus ashy, gray, gray-brown or brown, medulla I– 4
4. Epihymenium and exciple containing crystals dissolved in K .... **R. cinereovirens**
4. Epihymenium and exciple not containing crystals 5
5. Thallus containing stictic acid; ascospores 22–27 × 9–12 μm .... *R. hochstetteri*
5. Thallus containing no lichen substances 6
6. Exciple K+ violet; ascospores 14–17 × 6–9 μm .... *R. expallescens*
6. Exciple K–, ascospores 17.5–20 × 8–11 μm .... **R. infernulum f. sylvaticum**
7. Medulla I+ blue, ascospores usually <20 μm long 8
7. Medulla I–, ascospores usually >20 μm long 9
8. Epihymenium brown-black; hymenium hyaline to pale brown,
ascospores olive-green or brown, 12.5–16 × 6.5 μm .... *R. simillimum*
8. Epihymenium black; hymenium hyaline below, upper part pale red,
ascospores brown, 12.5–17.5 × 6–7 μm .... *R. umense*
9. Epihymenium olive-brown to green-black, K–; containing norstictic acid or
stictic acid, ascospores blue-green, 18–26 × 8–13 μm .... *R. copelandii*
9. Epihymenium red-brown to black brown, K+ violet 10
10. Exciple with crystals dissolved in K 11
10. Exciple without crystals 12
11. Exciple K-; ascospores 28–38 × 12–l8 μm .... *R. cinereonigrum*
11. Exciple K+ violet; ascospores 18–23 × 11.5–15 μm .... **R. rittokense**
12. Thallus pale gray-brown to brown, containing no lichen substances; ascospores
olive-brown to red-brown or brown, 25–35 × 12–15 μm .... *R. badioatrum*
12. Thallus gray to dark brown, containing gyrophoric acid;
ascospores olive-brown, 35–40 × 10–15 μm .... *R. sinense*
13. Asci 1- or 2-spored 14

13. Asci 8-spored 17
14. Asci 1-spored 15
14. Asci 2-spored 16
15. Ascospores green-brown to dark-brown .... *R.disporum*
15. Ascospores dark-green .... *R.solitarium*
16. Thallus gray, pruinose, containing norstictic acid and stictic acid;
epihymenium with crystals dissolved in K; ascospores dark green,
35–65 × 15–30 μm .... *R. geminatum*
16. Thallus dark grey, epruinose, containing barbatic acid; epihymenium
without crystals; ascospores hyaline, 37–55 × 20–25 μm .... *R. subgeminatum*
17. Ascospores submuriform, with 6–10 cells in optical section 18
17. Ascospores muriform 23
18. Thallus leprose .... *R. leprosulum*
18. Thallus areolate 19
19. Thallus containing stictic acid 20
19. Thallus containing no lichen substances 21
20. Medulla I+ blue, ascospores soon brown, 3-septate to submuriform,
20–27.5 × 7.5–12.5 μm .... *R. distinctum*
20. Medulla I-, ascospores persistently hyaline, submuriform,
25–30 × 15–18 μm .... *R. umbilicatum*
21. Prothallus distinct, black; ascospores dark green, submuriform,
20–25 × 12 μm .... *R. intersitum*
21. Prothallus absent or indistinct; ascospores hyaline 22
22. Hymenium vertically streaked red-brown, K+ violet; ascospores hyaline,
pale brown-green when over mature, 15–22.5 × 7.5–12.5 μm .... *R. subpostumum*
22. Hymenium hyaline, K–; ascospores persistently hyaline,
23–30 × 10.5–15 μm .... **R. roridulum**
23. Thallus ochraceous or rusty, epihymenium dark olive-brown,
ascospores hyaline, 24–34 × 14–16 μm .... *R. ischnothallum*
23. Thallus white, grey, brown 24
24. Ascospores <20 μm long (17–19 × 7–9 μm), hyaline,
pale-brown when over mature .... *R. cinereocaesium*
24. Ascospores >20 μm long 25
25. Ascospores soon becoming brown, 25–40 × 10–16 μm .... *R. grande*
25. Ascospores remaining hyaline or only finally darkening 26
26. Ascospores persistently hyaline 27
26. Ascospores hyaline, pale-brown when over mature 29
27. Thallus containing stictic acid .... *R. reductum*
27. Thallus containing no lichen substances 28
28. Epihymenium bluish green, K+ brighter;
ascospores 25–35 × 12.5–15 μm .... *R. sublavatum*
28. Epihymenium pale brown, or brown and olive-green intermixed, K–;
ascospores 31–40 × 12.5–13.5 μm .... *R. lavatum*

29. Medulla K–; ascospores 28–34 × 12–15 μm .... *R. gracile*
29. Medulla K+ yellow or red 30
30. Medulla K+ yellow, containing stictic acid .... *R. petraeum*
30. Medulla K+ red, containing norstictic acid .... *R. rubescens*

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MYCOTAXON

Echinoplaca infuscata sp. nov. and new records of the genus Echinoplaca from Yunnan, China

Meng-Li Zhu, Le-Wen Chen, Yan-Fei Zhao, Ze-Feng Jia*

College of Life Sciences, Liaocheng University
    Liaocheng 252059, Shandong Province, China

* Correspondence to: *zfjia2008@163.com*

Abstract—Three *Echinoplaca* spp. are reported from China. The new species *Echinoplaca infuscata* is characterised by pale brown apothecia having different morphologies and colors at different growth stages, and (3–)5-septate ascospores. *Echinoplaca epiphylloides* and *E. intercedens* are reported as new from China. The macromorphology, micromorphology, secondary chemistry, ecology, and distribution ranges of these three species are presented and discussed. A key is provided to the *Echinoplaca* species known in China.

Key words—foliicolous lichen, *Ascomycota*, *Ostropales*, *Gomphillaceae*, taxonomy

Introduction

Echinoplaca Fée (*Ascomycota, Ostropales, Gomphillaceae*) was established by Fée (1825), with *E. epiphylla* Fée as the type species. The genus was previously placed in *Asterothyriaceae* (Santesson 1952), but it was lateer found that *Echinoplaca* and *Gomphillus* (*Gomphillaceae*) had homology in hyphophore morphology (Vězda & Poelt 1987). More than 50 *Echinoplaca* species are known worldwide (Lücking 2008, Kondratyuk & al. 2013, Wijayawardene & al. 2017).

In southern China, foliicolous lichens are abundant, but *Echinoplaca* lacks detailed study with only seven species reported in China: *Echinoplaca* cf. *epiphylla*, *E. handelii* (Zahlbr.) Lücking, *E. hispida* Sipman, *E. leucotrichoides* (Vain.) R. Sant., *E. fusconitida* Lücking, *E. pellicula* (Müll. Arg.) R. Sant., and *E.*

tetrapla (Zahlbr.) Lücking (Aptroot & al. 2003, Zahlbruckner 1930, Santesson 1952, Aptroot & Sparrius 2003). Here, we propose *Echinoplaca infuscata* as a new species; and *E. epiphylloides* and *E. intercedens* are reported as new to China. An identification key is provided for the known species of *Echinoplaca* in China.

Materials & methods

Specimens, morphology, and chemistry

The specimens were collected from Yunnan, China, and deposited in the Fungarium of College of Life Sciences, Liaocheng University, Liaocheng, Shandong, China (LCUF). Dissecting microscope (Olympus SZX16) and light microscope (Olympus BX53) were used for the macro- and micromorphological studies. Measurements were taken from mature vertical sections of fruit bodies mounted in water. Secondary metabolites were examined by color test (10% KOH, saturated solution NaClO and p-phenylenediamine dissolved in ethanol) and thin-layer chromatography (TLC) using solvent C (Culberson 1972, Culberson & Kristinsson 1970).

DNA extraction, PCR sequencing, and phylogenetic analysis

Genomic DNA was extracted from ascomata of the specimens using the REDExtract-N-AmpTM Plant PCR kits (sigma-aldrich, U.S. and Canada) according to the manufacturer's protocol. The LSU and mtSSU regions were amplified using the primer pair AL2R/LR6 (Lumbsch & Schmitt 2002, Lumbsch & al. 2004) and mrSSU1/mrSSU3R (Zoller 1999). The 50 μL PCR reaction system contained 2 μL each primer solution (10 μmol/L), 2.0 μL genomic DNA, 19 μL ddH2O, and 25 μL 2×Taq PCR MasterMix (Tiangen, Beijing, China). Thermocycling conditions comprised initial denaturation at 95°C (5 min); 35 denaturation cycles at 94°C (45 s), annealing at 50°C (1 min), extension at 72°C (1.5 min); and a final extension at 72°C (10 min). The target product of PCR was assessed by electrophoresis on 1% agarose gels and sequenced by Biosune Inc. (Shanghai).

Contigs were assembled and edited using the program Geneious v6.1.2 (Biomatters Ltd., Auckland, NZ). The sequences from our new type specimen and GenBank data from 16 specimens of *Echinoplaca* spp. and an outgroup specimen of *Gyalectidium filicinum* (Amanda & al. 2022) were aligned using MAFFT v.7 (Katoh & Standley 2013). Multi-locus (mtSSU and LSU) phylogenetic analysis was performed. The combined analysis included 23 sequences (Table 1). The concatenated data matrix comprised 1479 nucleotide sites (LSU 552 bp and mtSSU 927 bp). Maximum likelihood (ML) and Bayesian inference (BI) were performed using the CIPRES Scientific gateway portal (http://www.phylo.org/portal2/) (Miller & al. 2010). Maximum likelihood bootstrapping analysis was performed, using the default parameters as implemented on the CIPRES, NSF XSEDE resource with bootstrap statistics calculated from 1000 bootstrap replicates (Stamatakis 2014). For the Bayesian analysis, the best substitution model was estimated using jModelTest 2.1.6 (Darriba & al. 2012). Based on the

results, we used GTR+I+G and GTR+G model. Bayesian analysis was performed using MrBayes 3.2.7a on CIPRES with 10 independent runs, searching for 10,000,000 generations with four independent chains and sampling every 1000th tree (Ronquist & Huelsenbeck 2003). After discarding the burn-in, the remaining 7500 trees of each run were pooled to calculate a 50% majority rule consensus tree. Generated phylogenetic tree was visualized and edited under Figtree v1.4.3.

Table 1. *Echinoplaca* and *Gyalectidium* sequences used in the analyses. Newly generated sequences are shown in bold.

Species	Specimen	Country	mtSSU	LSU
E. campanulata	Caceres & Lücking 168	Brazil	—	MZ851704
	Caceres & Lücking 171	Brazil	MZ827252	MZ851705
E. diffluens	Xavier-Leite 1901	Brazil	—	MZ851501
	Xavier-Leite 2001	Brazil	—	MZ851538
E. epiphylla	Caceres & Lücking 165a	Brazil	MZ827245	—
	Caceres & Lücking 165b	Brazil	MZ827246	—
E. infuscata	**YN210763c**	**China**	**ON360972**	ON117295
E. leucotrichoides	Xavier-Leite 1989	Brazil	MZ827184	MZ851534
	Xavier-Leite & Caceres 1734	Brazil	MZ827190	MZ851603
E. lucernifera	Lücking s.n., F	Costa Rica	AY341370	—
	Lücking 59c	Costa Rica	—	MZ851714
E. marginata	Xavier-Leite 1539	Brazil	—	MZ851489
	Xavier-Leite 1572b	Brazil	—	MZ851586
E. pellicula	Xavier-Leite 1502	Brazil	—	MZ851484
	Xavier-Leite 1560	Brazil	—	MZ851490
E. tetrapla	Xavier-Leite & al. 1422	Brazil	—	MZ851635
	Xavier-Leite & al. 1397a	Brazil	—	MZ851655
G. filicinum	Xavier-Leite 1571b	Brazil	MZ827278	MZ851584

Results

The phylogenetic trees obtained from maximum likelihood (ML) and Bayesian inference analysis (BI) exhibited the same topology. *Echinoplaca infuscata* obtained a high support value (97%/0.9935). The molecular phylogeny based on the mitochondrial large subunit ribosomal (LSU) and small subunit marker (mtSSU) of *Echinoplaca* exhibits a well-supported polyphyletic lineage (Amanda & al. 2022). *Echinoplaca infuscata* clustered with *E. pellicula*, but they can be distinguished easily in morphology.
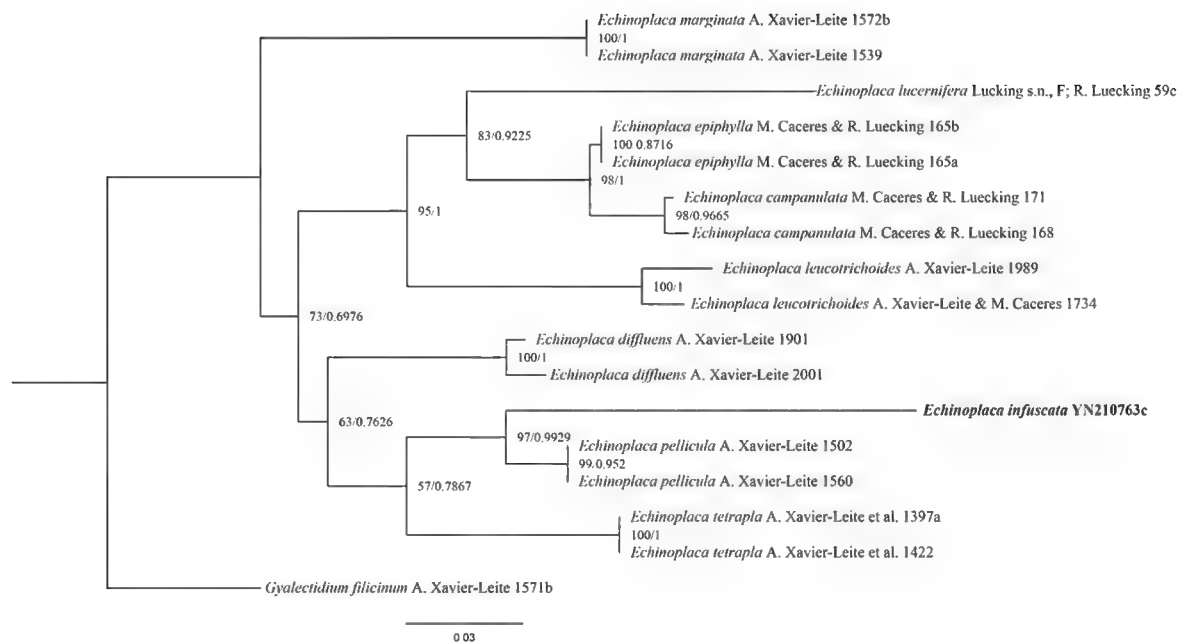
Fig. 1. The maximum likelihood (ML) phylogenetic tree generated from analysis of combined LSU and mtSSU of *Echinoplaca* spp., with *Gyalectidium filicinum* as outgroup. ML-bootstrap values/ Bayesian posterior probabilities are written next to nodes. Newly generated sequences are shown in bold.

Taxonomy

Echinoplaca infuscata M.L. Zhu & Z.F. Jia, **sp. nov.**   Fig. 2a–c

FN571018

Differs from *Echinoplaca tetrapla* by its brownish apothecia and (3–)5-septate ascospores.

Type: China. Yunnan Province, Mengla County, Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 21.9156°N 101.1869°E, alt. 640 m, on leaves, 1 Jul. 2021, M.L. Zhu YN210763c (Holotype, LCUF; GenBank ON117295, ON360972).

Etymology: The specific epithet *infuscata* refers to the brownish apothecia.

Thallus foliicolous, crustose, continuous, pale green to green, with corticiform layer, finely white verrucose due to incrustation with calcium oxalate crystals, with scattered, sterile setae; verrucae 0.05–0.1 mm in diam., setae 0.5–1.0 mm long, white to pale brown. Photobiont a species of *Trebouxia*. Ascomata apothecia, adnate, spot-like, emarginate, rounded, 0.3–0.6 mm in diam.; disc plane, pale brown, often having different morphologies and colors at different growth stages, emarginate and darker brown initially, plane and pale brown when mature. Excipulum colorless to pale brown, 20–55 μm wide; epithecium pale brown, 4–10 μm high; hymenium colorless, 30–55 μm high; hypothecium pale brown, 6–18 μm high. Asci 4–8-spored, clavate, 26–44 × 9–24 μm, I–. Ascospores ellipsoid, (3–)5-septate, with slight constrictions at

septa, 12–17 × 5–7 μm, about 2.5–3 times as long as broad. Hyphophores not observed.

Chemistry—Thallus K–, C–, KC–, P–. No substances detected by TLC.

Ecology & distribution—On the leaves in tropical rainforest of Yunnan Province, China.

> Additional specimens examined: **CHINA. Yunnan Province, Mengla County,** Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 1 Jul. 2021 M.L. Zhu (LCUF YN210764b, YN210765a, YN210773a, YN210776a, YN210853b, YN210881a, YN210896).

Comments—*Echinoplaca infuscata* is characterised by its pale green thallus, sterile setae sometimes present, white to pale brown; pale brown apothecia; and (3–)5-septate ascospores. *Echinoplaca tetrapla* and *E. intercedens* are closely related to *E. infuscata,* all of them having ascospores with 3(–5) septa, but *E. tetrapla* has greyish brown to brownish black, slightly shiny apothecia, *E. intercedens* often has dispersed thallus, and chocolate-brown apothecia (Lücking & al. 2001, Lücking 2008).

Echinoplaca epiphylloides Lücking, Fl. Neotrop., Monogr. 103: 476. 2008.

Fig. 2d–f

Thallus foliicolous, crustose, continuous, pale greenish grey, irregular in outline, with cartilaginous, corticiform layer, finely verrucose and numerous sterile setae; verrucae 0.05–0.1 mm in diam., white; setae 0.6–1 mm long, white. Photobiont a species of *Trebouxia.* Ascomata apothecia, adnate, spot-like, emarginate, rounded, 0.4–0.6 mm in diam.; disc plane, pale yellow. Excipulum colorless, 20–28 μm wide; epithecium pale brown, 5–10 μm high; hymenium colorless, 40–50 μm high; hypothecium colorless to pale brown, 6–12 μm high. Asci 4–8-spored, clavate, 40–60 × 10–15 μm, I–. Ascospores fusiform to oval, 5–7-septate, with constrictions at septa, 18–30 × 4–8 μm, about 3.5–4.5 times as long as broad. Hyphophores not observed.

Chemistry—Thallus K–, C–, KC–, P–. No substances detected by TLC.

> specimens examined: **CHINA. Yunnan Province, Mengla County,** Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 1 Jul. 2021, M.L. Zhu (LCUF YN210763a, YN210771a, YN210816, YN210822b, YN210878a, YN210894, YN210987, YN211018b, YN211099).

Distribution—Ecuador, Guyana, and Brazil (Lücking 2008).

Comments—The species is reported from South America usually produces ascospores mainly with five septa, and filiform diahyphae with terminal segments narrowly fusiform (Lücking 2008). In Chinese material, we find

this species usually produces ascospores with 5–7 septa, and diahyphae were not found. *Echinoplaca pellicula* is similar to *E. epiphylloides* in the color of apothecia, but differs by its sparse sterile setae (Lücking 2008).

Echinoplaca intercedens Vězda, Acta Mus. Silesiae, Ser. A, 22: 83. 1973.

Fig. 2G–I

THALLUS foliicolous, crustose, continuous or disperse, pale gray-green to slightly white, with corticiform layer, fine verrucae and sterile setae; verrucae 0.05–0.15 μm in diam., setae pale brown, 0.1–0.3 mm long. PHOTOBIONT a species of *Trebouxia*. ASCOMATA apothecia, adnate, spot-like, emarginate, rounded, 0.4–0.7 mm in diam.; disc plane, brown to dark brown. EXCIPULUM colorless to pale brown, 15–30 μm wide; EPITHECIUM brown, 6–12 μm high; HYMENIUM colourless, 45–65 μm high; HYPOTHECIUM colorless, 7–15 μm high. ASCI 4(–8)-spored, clavate, 27–48 × 10–20 μm, I–. ASCOSPORES ovoid, 3–5-septate, with constrictions at septa, 11–18 × 4–6 μm, 2.5–3.5 times as long as broad. HYPHOPHORES not observed.

CHEMISTRY—Thallus K–, C–, KC–, P–. No substances detected by TLC.

SPECIMEN EXAMINED: **CHINA. YUNNAN PROVINCE, Mengla County,** Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 1 Jul. 2021, M.L. Zhu (LCUF YN210829, YN210851b, YN210872a, YN210886, YN210988, YN210998b, YN211008, YN211019a, YN211068b, YN211069, YN211105b, YN211108a, YN211109).

DISTRIBUTION—U.S.A., Colombia, Ecuador, Brazil, and Bolivia (Lücking 2008).

COMMENTS—Our specimens conform to those described by Lücking (2008), which are characterised by its apothecia brown to dark brown and matt, and 3–5-septate ascospores. The most similar species is *E. triseptata*, which differs by having apothecia sordid to purplish brown, thallus usually with thin, white pruina, and ascospores 3-septate (Lücking 2008).
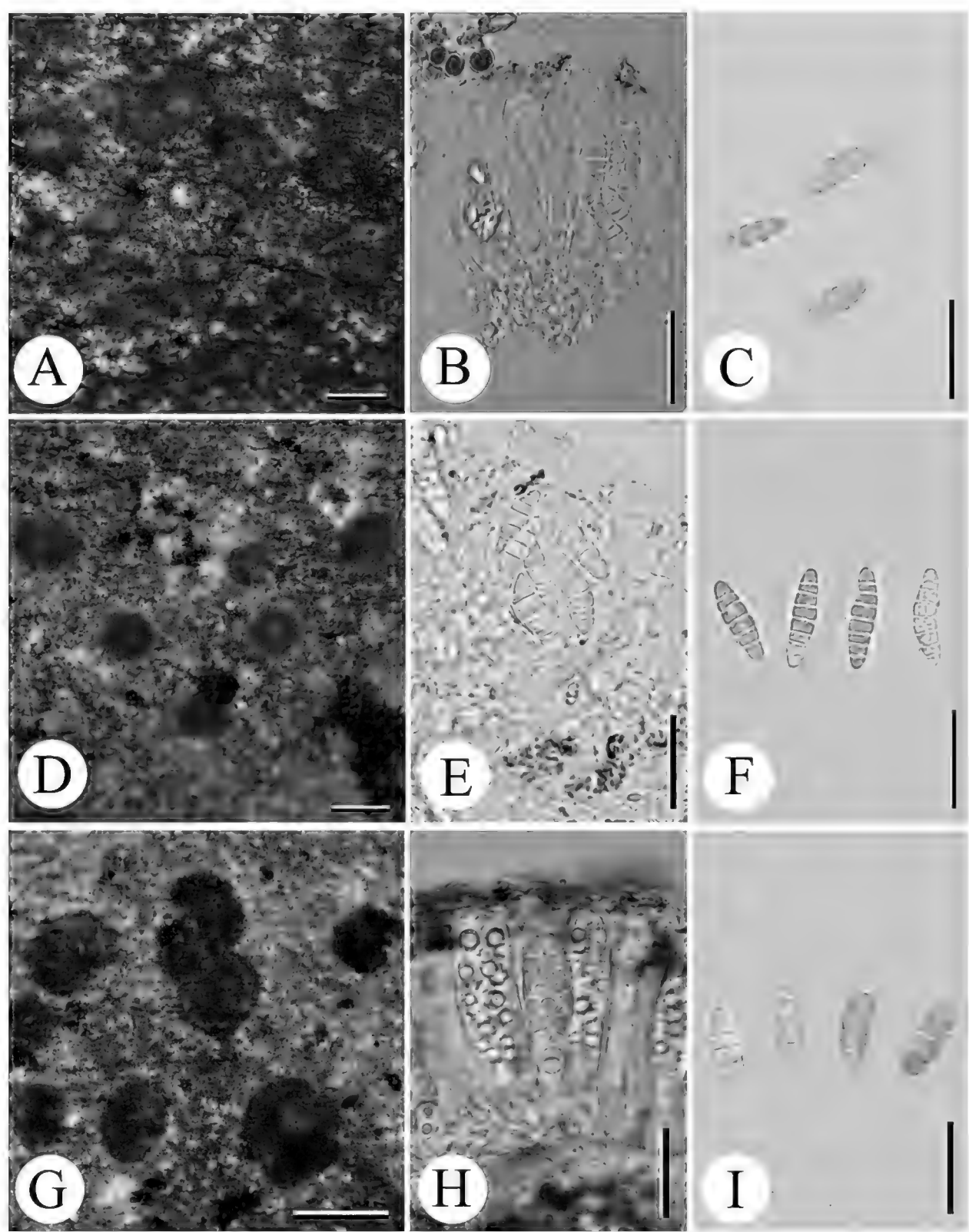
FIG. 2. Habitus, asci and ascospores of *Echinoplaca* spp. A–C: *Echinoplaca infuscata* (LUCF, Zhu YN210763c); D–F: *Echinoplaca epiphylloides* (LUCF, Zhu YN210987); G–I: *Echinplaca intercedens* (LUCF, Zhu YN210829)

Key to the species of *Echinoplaca* known from China

1. Ascospores (sub-)muriform 2
1. Ascospores transversely septate 4
2. Ascospores no more than 30 μm long .... *E. handelii*
2. Ascospores more than 30 μm long 3
3. Apothecia dark greyish brown to blackish brown .... *E. fusconitida*
3. Apothecia pale yellow to orange .... *E.* cf. *epiphylla*
4. Ascospores 15–27-septate, 65–100 × 11–18 μm; apothecia 0.4–0.8 mm diam;
thallus finely verrucose .... *E. leucotrichoides*
4. Ascospores 3–7-septate 5
5. Thallus with sparse sterile setae; apothecia pale yellow to brown; ascospores
(3–)5(–7)-septate, 12–26 ×5–9 μm .... *E. pellicula*
5. Thallus with abundant sterile setae 6
6. Apothecia pale yellow to orange to brownish yellow 7
6. Apothecia brown 8
7. Apothecia pale yellow to orange; setae white .... *E. epiphylloides*
7. Apothecia orange to brownish yellow; setae pale brownish .... *E. hispida*
8. Apothecia pale brown; setae 0.5–1.0 mm long, white to pale brown; ascospores
12–17 × 5–7 μm .... *E. infuscata*
8. Apothecia dark brown or greyish brown 9
9. Setae 0.6–1.2 mm long; ascospores 11–20 × 4–6 μm .... *E. tetrapla*
9. Setae 0.1–0.3 mm long; ascospores 13–21 × 4–6 μm .... *E. intercedens*

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Pestalotiopsis sonneratiae sp. nov. from China

Ning Jiang[1], Long-Yan Tian[2], Han Xue[1], Chun-Gen Piao[1], Yong Li[1*]

[1] *Key Laboratory of Forest Protection of National Forestry and Grassland Administration, Ecology and Nature Conservation Institute, Chinese Academy of Forestry, Beijing 100091, China*
[2] *Guangdong Provincial Key Laboratory of Silviculture, Protection and Utilization, Guangdong Academy of Forestry, Guangzhou 510520, China*
[*] Correspondence to: *lylx@caf.ac.cn*

Abstract—A new pestalotioid species, *Pestalotiopsis sonneratiae*, was isolated from leaf spot symptoms of *Sonneratia apetala* (mangrove apple) in Guangdong Province, China. The pathogen was characterized by morphological and molecular data ITS, tef1, and tub2 loci. The species is described, illustrated, and compared with similar *Pestalotiopsis* species and another pestalotioid pathogen of *Sonneratia*.

Key words—*Amphisphaeriales,* phylogeny, *Sporocadaceae,* taxonomy

Introduction

Pestalotioid fungi are easily characterized by owning multi-septate and more or less fusiform conidia with appendages at one or both ends, frequently with some melanised cells (Liu & al. 2019). *Pestalotiopsis* Steyaert (*Sporocadaceae, Amphisphaeriales*) is a typical pestalotioid genus with 5-celled conidia, separated from *Pestalotia* with 6-celled conidia (Steyaert 1949). With molecular evidence, Maharachchikumbura & al. (2014) split *Pestalotiopsis* s.lat. into *Neopestalotiopsis, Pseudopestalotiopsis,* and *Pestalotiopsis* s.str. Subsequently, several new species were proposed based on morphology and phylogeny of combined ITS, tef1, and tub2 loci (Gu & al. 2021, Liu & al. 2017, Norphanphoun & al. 2019).

Pestalotiopsis s.lat. is widely distributed in tropical and temperate regions, usually inhabiting plants as endophytes, pathogens and saprophytes (Ismail

& al. 2013, Maharachchikumbura & al. 2014). Several species are associated with economic plant diseases, for example, *Neopestalotiopsis rosicola* C.M. Tian & N. Jiang causes stem canker of *Rosa chinensis* (Jiang & al. 2018); *N. vitis* is the causal agent of *Vitis vinifera* (grapevine) leaf spot (Jayawardena & al. 2016); *Pestalotiopsis kenyana* Maharachch. & al. results in *Castanea mollissima* (Chinese chestnut) leaf disease (Jiang & al. 2021); several *Pestalotiopsis* species cause *Camellia oleifera* leaf blight (Li & al. 2021).

Sonneratia apetala Buch.-Ham. (*Lythraceae*; mangrove apple) is a main species of mangrove forests in southern China (Ren & al. 2009). Frequent brown leaf spots were discovered during our investigation of *S. apetala* diseases in Guangdong Province in China (Fig. 1). Here we propose the fungal pathogen as a new species, *Pestalotiopsis sonneratiae*, based on modern taxonomic methods of morphology and phylogeny.

Fig. 1. Leaf spots on *Sonneratia apetala*.

Materials & methods

Samples and isolates

In this study, diseased mangrove apple leaves were collected, packed in paper bags and transferred to the laboratory for fungal isolation. The symptomatic leaves were primarily surface-sterilized for 1 min in 75% ethanol, 3 min in 1.25% sodium hypochlorite, and 1 min in 75% ethanol, then rinsed for 2 min in distilled water and blotted on dry sterile filter paper. Then the diseased areas of the leaves were cut into 0.5 × 0.5 cm pieces using an aseptic razor blade, and transferred onto the surface of potato dextrose agar (PDA; 200 g potatoes, 20 g dextrose, 20 g agar per L) and malt extract agar (MEA; 30 g malt extract, 5 g mycological peptone, 15 g agar per L) plates, and incubated at 25°C to obtain pure cultures. The cultures were deposited in the China Forestry Culture Collection Center, Ecology and Nature Conservation Institute,

Chinese Academy of Forestry, Beijing, China (CFCC; http://www.cfcc-caf.org.cn/), and the specimens in the herbarium of the Chinese Academy of Forestry, Beijing, China (CAF; http://museum.caf.ac.cn/).

Morphological studies

Species identification was based on conidial morphology formed on PDA plates. Fifty conidia were selected randomly for measurement using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with a Nikon digital sight DS-Ri2 camera. Cultural characteristics were observed on PDA and MEA after 10 days incubation at 25°C in the dark.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fungal mycelium growing on MEA using a CTAB method (Doyle & Doyle 1990). The internal transcribed spacer of rDNA (ITS) was amplified using primers ITS1 and ITS4 (White & al. 1990). The translation elongation factor-1 alpha (tef1) was amplified using primers EF1-728F and EF2 (Carbone & Kohn 1999). The β-tubulin gene (tub2) was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). These regions were amplified as follows: an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 50 s at 52°C (ITS) or 54°C (tef1 and tub2), and 1 min at 72°C, and a final elongation step of 7 min at 72°C. The PCR products were estimated visually by electrophoresis in 2% agarose gel set to 60 V for 90 min. DNA was sequenced using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminater Kit v. 3.1 at the Shanghai Invitrogen Biological Technology Company Limited.

DNA sequence analysis

The new sequences generated in this study and the reference sequences of *Pestalotiopsis* species included in the phylogenetic analyses are summarized in Table 1. *Neopestalotiopsis magna* (MFLUCC 12-0652) was used as the outgroup taxon. These sequences were aligned with MAFFT v. 7 (Katoh & Standley 2013) and manually adjusted. Phylogenetic analyses were generated from the combined ITS–tef1–tub2 sequences using PhyML v. 3.0 (Guindon & al. 2010) for maximum Likelihood (ML), and MrBayes v.3.1.2 for Bayesian Inference (BI) (Ronquist & Huelsenbeck 2003).

Results

Molecular phylogeny

The combined ITS, tef1, and tub2 alignment comprised 45 sequences (including one outgroup taxon) and 1479 characters including alignment gaps, of which 1102 were parsimony informative, 240 were variable and parsimony-uninformative, and 137 were constant. The topologies resulting from ML and BI analyses of the concatenated dataset were congruent (Fig. 2). The new species appeared in a single clade with high bootstrap support (Fig. 2).

TABLE 1. Sequences of *Neopestalotiopsis* and *Pestalotiopsis* used in the molecular analysis.

SPECIES	ISOLATE	GenBank accessions numbers		
		ITS	tub2	tef1
N. magna	MFLUCC 12-0652*	KF582795	KF582793	KF582791
P. abietis	CFCC 53011*	MK397013	MK622280	MK622277
	CFCC 53012	MK397014	MK622281	MK622278
P. australasiae	CBS 114126*	KM199297	KM199409	KM199499
	CBS 114141	KM199298	KM199410	KM199501
P. biciliata	CBS 124463*	KM199308	KM199399	KM199505
	CBS 236.38	KM199309	KM199401	KM199506
P. brachiata	LC2988*	KX894933	KX895265	KX895150
	LC8188	KY464142	KY464162	KY464152
	LC8189	KY464143	KY464163	KY464153
P. camelliae-oleiferae	CSUFTCC08*	OK493593	OK562368	OK507963
	CSUFTCC09	OK493594	OK562369	OK507964
P. disseminata	CBS 143904	MH554152	MH554825	MH554587
	MEAN 1165	MT374687	MT374712	MT374699
P. dracontomelonis	MFLU 14-0207*	KP781877	—	KP781880
P. ericacearum	IFRDCC 2439*	KC537807	KC537821	KC537814
P. etonensis	BRIO 66615*	NR_145237	KM199405	KM199509
P. formosana	NTUCC 17-009*	MH809381	MH809385	MH809389
P. grevilleae	CBS 114127*	KM199300	KM199407	KM199504
P. kenyana	CBS 442.67*	KM199302	KM199395	KM199502
P. knightiae	CBS 111963	KM199311	KM199406	KM199495
	CBS 114138*	KM199310	KM199408	KM199497
P. macadamiae	BRIP 63738b	KX186588	KX186680	KX186621
	BRIP 63739b	KX186587	KX186679	KX186620
P. nanjingensis	CSUFTCC16*	OK493602	OK562377	OK507972

Species	Isolate	GenBank accessions numbers		
		ITS	tub2	tef1
P. nanningensis	CSUFTCC10*	OK493596	OK562371	OK507966
P. oryzae	CBS 171.26	KM199304	KM199397	KM199494
	CBS 353.69*	KM199299	KM199398	KM199496
P. parva	CBS 265.37	KM199312	KM199404	KM199508
	CBS 278.35*	KM199313	KM199405	KM199509
P. photiniicola	GZCC 16-0028*	KY092404	KY047663	KY047662
P. rhizophorae	MFLUCC 17-0416*	MK764283	MK764349	MK764327
P. rhodomyrti	HGUP4230*	KF412648	KF412642	KF412645
P. sonneratiae	**CFCC 57392**	**ON114182**	**ON086814**	**ON086810**
	CFCC 57393	**ON114183**	**ON086815**	**ON086811**
	CFCC 57394*	**ON114184**	**ON086816**	**ON086812**
	CFCC 57395	**ON114185**	**ON086817**	**ON086813**
P. telopeae	CBS 114137	KM199301	KM199469	KM199559
	CBS 114161*	KM199296	KM199403	KM199500
	CBS 113606	KM199295	KM199402	KM199498
P. terricola	CBS 141.69*	MH554004	MH554680	MH554438
P. thailandica	MFLUCC 17-1616*	MK764285	MK764351	MK764329
P. trachycarpicola	OP068*	JQ845947	JQ845945	JQ845946
	IFRDCC 2403	KC537809	KC537823	KC537816
	LC4523	KX895011	KX895344	KX895230

Ex-type strains are marked with *.
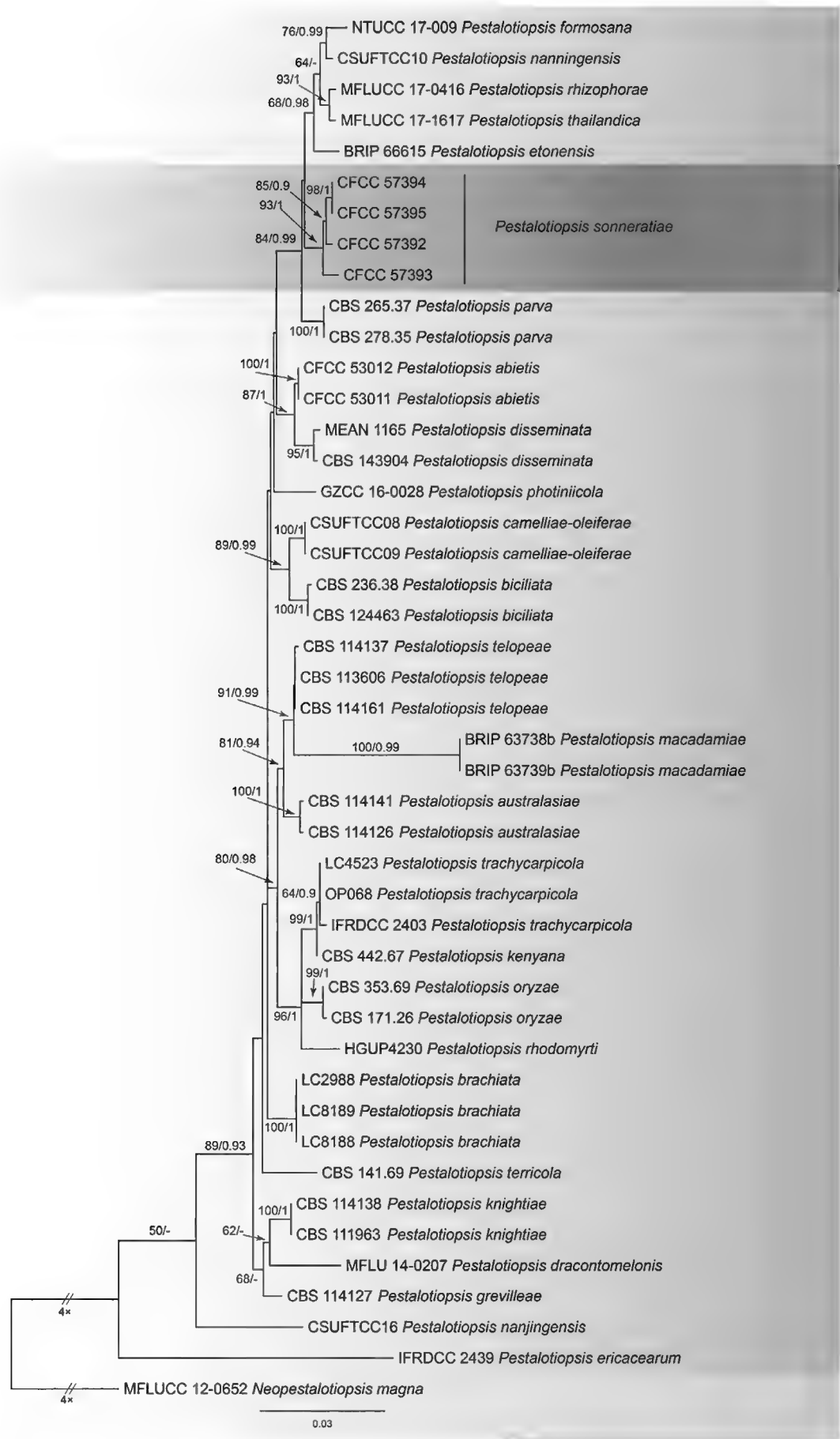
FIG. 2. Phylogram of *Pestalotiopsis* resulting from a maximum likelihood analysis based on a combined matrix of ITS, tef1, and tub2 loci. Numbers above the branches indicate ML bootstrap values (left, ML BS ≥50%) and Bayesian posterior probabilities (right, BPP ≥0.9). The tree is rooted with *Neopestalotiopsis magna* (MFLUCC 12-0652). Isolates from the present study are marked in blue.

Taxonomy

Pestalotiopsis sonneratiae Ning Jiang, sp. nov.   Fig. 3

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Differs from phylogenetically close *Pestalotiopsis etonensis, P. formosana, P. nanningensis, P. rhizophorae,* and *P. thailandica* by its host *Sonneratia* (*Lythraceae, Malpighiales*) and by sizable differences in its ITS, tef1, and tub2 sequences.

TYPE: China, Guangdong Province, Zhongshan City, Hengmen village, 22.8056°N 113.9694°E, on leaves of *Sonneratia apetala*, 26 September 2018, coll. L.Y. Tian JNH0045 (**Holotype,** CAF800049; ex-type culture CFCC 57394; GenBank ON114184, ON086816, ON086812).

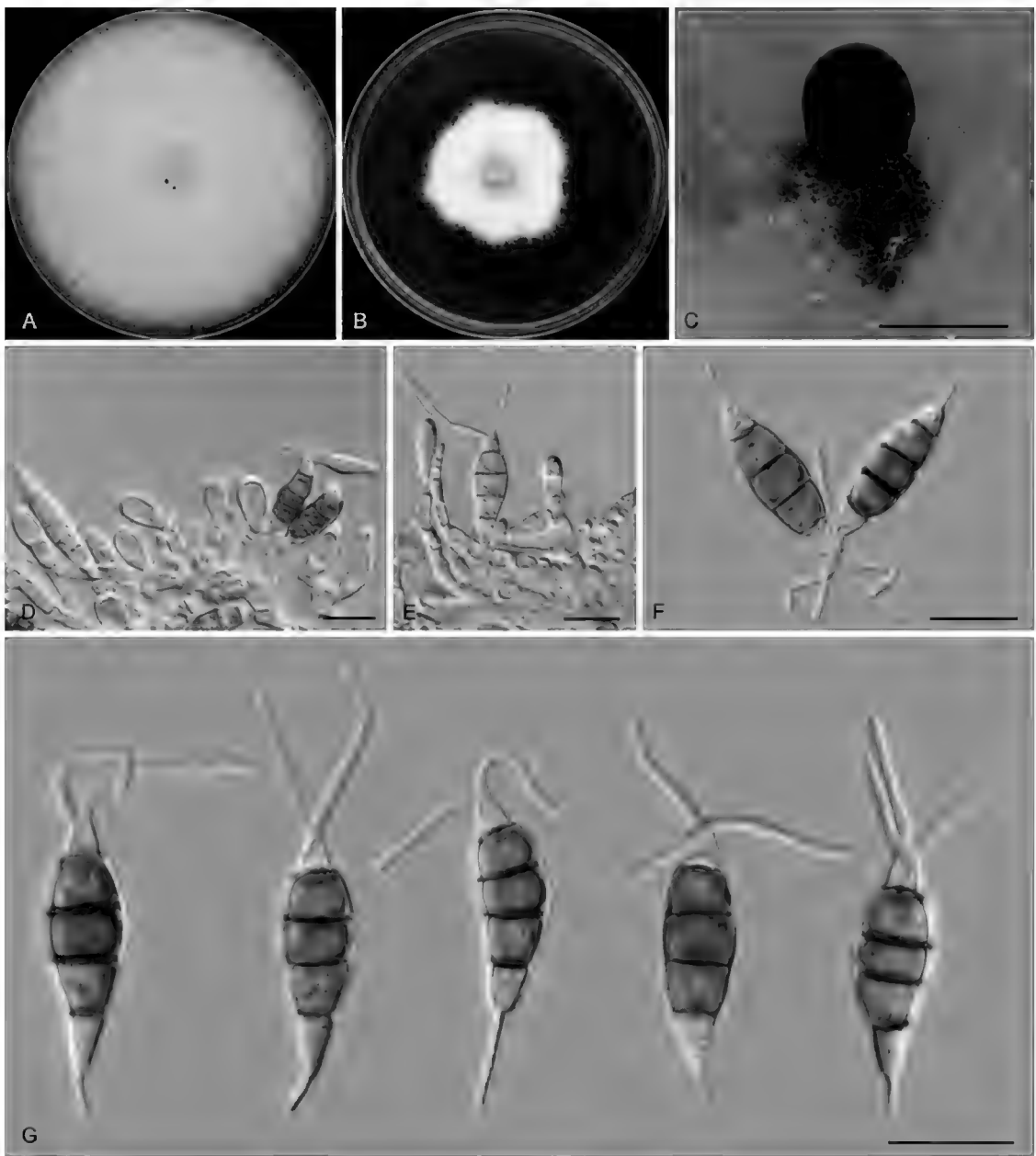ETYMOLOGY: *sonneratiae,* named after the host genus, *Sonneratia.*

FIG. 3. *Pestalotiopsis sonneratiae* (ex-holotype, CFCC 57394). A. Colony on PDA after 10 d at 25°C; B. Colony on MEA after 10 d at 25°C; C. Conidioma formed on PDA; D, E. conidiogenous cells giving rise to conidia; F, G. conidia. Scale bars: C = 300 μm; D–G = 10 μm.

Sexual morph: Undetermined. Asexual morph: Conidiomata acervular, aggregated or solitary, erumpent, pulvinate, black, 100–350 μm diam., exuding black conidial masses. Conidiophores indistinct, usually reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, cylindrical to ampulliform, annelidic, 7.5–36.5 × 1.5–5 μm, means ± SD = 16 ± 1.9 × 3.5 ± 0.7 μm. Conidia fusoid, straight or slightly curved, 4-septate, smooth, slightly constricted at the septa, (16–)17.5–20.5(–22) × (5.5–)6–8(–8.5) μm, means ± SD = 19.1 ± 1.4 × 6.5 ± 1.6 μm (n = 50), L/W = 2.5–3.5; basal cell obconic with a truncate base, thin-walled, hyaline or pale brown, (3–)3.5–5(–5.5) μm; median cells 3, trapezoid or subcylindrical, concolourous, brown, thick-walled, the first median cell from base (3–)3.5–5 μm long, the second cell (3–)3.5–4(–4.5) μm long, the third cell (3–)3.5–4.5(–5) μm long, together (10.5–)11–13(–14) μm long; apical cell conic with an acute apex, thin-walled, hyaline, (2–)2.5–3.5(–4) μm long; basal appendage single, unbranched, tubular, centric, straight or slightly bent, 2.5–5(–8) μm long, mean ± SD = 3.8 ± 1.3 μm; apical appendages 2–3, unbranched, tubular, centric, straight or bent, (8–)10–15.5(–18) μm long, mean ± SD = 12.7 ± 2.4 μm. Colonies on MEA flat, with undulate edge, white to sienna, reaching 40 mm diam after 10 d at 25°C; on PDA flat, spreading, with flocculent aerial mycelium and entire edge, white, reaching 90 mm diam after 10 d at 25°C, forming black conidiomata with black conidial masses.

Additional specimen examined: **CHINA, Guangdong Province, Zhongshan City,** Hengmen village, 22.8056°N 113.9694°E, on leaves of *Sonneratia apetala*, 8 June 2019, coll. L.Y. Tian (CFCC 57395; GenBank ON114185, ON086817, ON086813); (CFCC 57392; GenBank ON114182, ON086814, ON086810); (CFCC 57393; GenBank ON114183, ON086815, ON086811).

Host/Distribution: on leaves of *Sonneratia apetala* in China.

Note: The four isolates of *Pestalotiopsis sonneratiae* clustered in a well-supported clade (ML/BI = 93%/1) in Fig. 2 and appeared closely related to *P. etonensis*, *P. formosana*, *P. nanningensis*, *P. rhizophorae*, and *P. thailandica*. Morphologically, *P. sonneratiae* is similar to them in conidial size, but is found on a host in a different botanical family and order (Table 2; Ariyawansa & al. 2018, Crous & al. 2020, Li & al. 2021, Norphanphoun & al. 2019). Phylogenetically, *P. sonneratiae* can be distinguished by sequence data (nucleotide differences from *P. etonensis* in ITS: 3/505; tef1: 6/483; tub2: 11/447; from *P. formosana* in ITS: 2/499; tef1: 8/482; tub2: 9/445; from *P. nanningensis* in ITS: 8/505; tef1: 28/480; tub2: 26/442; from *P. rhizophorae* in ITS: 6/505; tef1: 6/476; tub2: 13/442; from *P. thailandica* in ITS: 5/506; tef1: 6/476; tub2: 12/442).

TABLE 2. Morphological comparison of *Pestalotiopsis sonneratiae* and related species.

SPECIES	HOST	CONIDIAL SIZE (μm)	BASAL APPENDAGE LENGTH (μm)	NO. APICAL APPENDAGES	APICAL APPENDAGE LENGTH (μm)	REFERENCE
P. etonensis	*Sporobolus jacquemontii (Poaceae)*	15–21 × 4–7	2–4.5	3	6–16	Crous & al. 2020
P. formosana	— *(Poaceae)*	18–22 × 6–7	3–5	2	11–16	Ariyawansa & al. 2018
P. nanningensis	*Camellia oleifera (Theaceae)*	24–26.5 × 7–8	4.5–6.5	2–3	18–22.5	Li & al. 2021
P. rhizophorae	*Rhizophora apiculata (Rhiozphoraceae)*	17.5–23 × 6–6.5	1.5–4.5	1–2	8–13	Norphanphoun & al. 2019
P. sonneratiae	*Sonneratia apetala (Lythraceae)*	16–22 × 5.5–8.5	2.5–8	2–3	8–18	This study
P. thailandica	*Rhizophora apiculata (Rhiozphoraceae)*	17.5–28 × 5.5–6.5	2.5–9.5	1–2	11–34	Norphanphoun & al. 2019

Discussion

In this study, disease samples with typical leaf spot symptom were observed and collected from *Sonneratia apetala* in Guangdong Province in China. Based on DNA sequence data of combined ITS, tef1, and tub2 loci, *Pestalotiopsis sonneratiae* is proposed here as the potential pathogen causing *S. apetala* leaf spot disease.

Another pestalotioid species *Neopestalotiopsis sonneratiae* Norph. & al. [as "*sonneratae*"] was reported to be associated in Thailand with leaf spots on *Sonneratia alba* Sm. [Norphanphoun & al. 2019; as "*Sonneronata*"]. However, our *P. sonneratiae* is obviously distinguished from *N. sonneratiae* by pigmentation in the three median cells of the conidia (Norphanphoun & al. 2019).

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Tricholoma punctatum sp. nov. from Serbia

NEBOJŠA LUKIĆ[1], ELEONORA ČAPELJA[2], MAJA KARAMAN[2]

[1] *Mycological Society "Šumadija"*
        Daničićeva 120/36, 34000 Kragujevac, Serbia
[2] *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology*
        Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia
*CORRESPONDENCE TO: *eleonora.capelja@dbe.uns.ac.rs*

ABSTRACT–A new species of *Tricholoma* recorded from a temperate forest in central Serbia is described and illustrated. It is characterized by the presence of punctate, often elevated, almost black spots arranged in one to three circular zones on the pileus. The new species showed morphological differences from closely related taxa and molecular phylogenetic analysis of ITS and LSU regions confirm the distinct taxonomic status of the finding.

KEY WORDS: *Atrosquamosa, Tricholomataceae*, taxonomy, fungal diversity

Introduction

Tricholoma (Fr.) Staude (*Tricholomataceae, Agaricales*) is a cosmopolitan genus, mainly distributed in temperate and subtropical zones of both hemispheres (Tedersoo & al. 2010). The majority of *Tricholoma* species are described from North America where more than one hundred species have been reported so far, and this region appears to be the centre of species richness (Bessette & al. 2013). There are about ninety species listed from Europe (Bon 1991, Riva 1988, 1998, 2003; Kibby 2010), but the overall diversity of *Tricholoma* taxa in Europe is still poorly resolved.

Serbia presents an exceptional place for mycological research due to the presence of various habitats: thermophilous oak forest at lower altitudes (usually *Quercus cerris* L.); succeeded by mesophilous beech and coniferous forests (usually *Fagus sylvatica* L.; *Picea abies* (L.) H. Karst.; *Abies alba* Mill.) in

higher altitudes. Pine forests can be sporadically found at all altitudes. Although they are some published data about diversity of *Agaricomycetes* in Serbia (Čolić 1967, Karaman & al. 2005, Karadžić & Milijašević 2008, Lukić 2009, 2013; Ivančević & Davidović 2011, Sadiković & al. 2012), a thorough and systematic investigation of this group of fungi is still lacking. *Tricholoma* is quite frequent in Serbia (Uzelac 2009), with basidiomes often appear in late October and November in thermophilous deciduous forests. Since *Tricholoma* species show limited microscopic variation, and are characterized by hyaline, subglobose to oblong spores, simple pileipellis structures, and lack of well-differentiated sterile elements (e.g., cystidia), species identification and partly also the infrageneric classification have mainly been based on macromorphology. In a recent work, the combination of morphological and molecular data showed that characters such as pileus color, pileipellis structure, presence of clamp connections and spore size seem to be rather conserved within accepted sections, while the presence of a distinct ring, and especially host specificity were highly variable (Heilmann-Clausen & al. 2017).

The first comprehensive molecular work on European *Tricholoma* (Heilman-Clausen & al. 2017) revealed ten clades/sections which were not completely in agreement with Singer's classification. Also, they considered European species of sections *Caligata*, *Atrosquamosa*, and *Terrea* to be well evaluated taxonomically, while for all other sections their sampling was limited and are in need for further phylogenetic studies. Heilman-Clausen & al. (2017) discovered several clades with unassigned names, and one of them included two European species *T. josserandii* Bon and *T. fucatum* (Fr.) P. Kumm., taxa that were grouped together with North and Central American species described and mentioned in study by Ovrebo & al. (2019). A phylogenetic analysis by Reschke & al. (2018) of *Tricholoma* collections from Asia, Europe, and North America revealed major clades that were similar to those presented by Christensen & Heilman-Clausen (2013). Also, sequences of *T. imbricatum* (Fr.) P. Kumm. specimens from USA formed a highly supported clade that was separated from the Asian/European *T. imbricatum* clade. Additionally, *T. aurantiipes* Hongo, *T. davisiae* Peck, *T. muscarium* Kawam. ex Hongo, and *T. muscarioides* Reschke & al. comprised a clade that was formally described as a new section *Muscaria*. All of the preceding analyses were based only on ITS sequences, which has proved to be a stable marker for species delimitations; but this region cannot resolve higher taxonomic relationships at a sufficiently detailed level (Frøslev & al. 2005). Infrageneric classification proposed in previous studies should be viewed as preliminary and further studies including multiple molecular markers should

be conducted. Here we present Serbian specimens with unique morphological characters differing from previous *Tricholoma* species recorded from Europe (Riva 1988, 1998, 2003); and phylogenetic analyses based on ITS and LSU confirm these specimens as a new species, proposed here as *T. punctatum*.

Material and methods

Studied material

Specimens studied for this paper were collected by the first author during field trips in central Serbia during October–November 2020. Nearly one hundred basidiomes were recorded during three visits at the defined location (close to the city of Kragujevac, 44.0181°N 20.8839°E, altitude 240 m). The habitat of the newly proposed *Tricholoma* species (widely observed) is an old oak forest (mostly *Quercus robur* L. and *Q. cerris*) with several other interspersed trees (*Pinus nigra* J.F. Arnold, *P. sylvestris* L., *Carpinus betulus* L., *Tilia cordata* Mill., *Populus alba* L., *Betula pendula* Roth) within the Memorial area of Šumarice near the city of Kragujevac with an approximate area of 350 ha. By virtue of the forest age and absence of economic exploitation the defined area represents a very valuable mycological habitat. During twenty years of research in this area twenty-one *Tricholoma* species (and several varieties and forms) have been found mostly within sections *Genuina, Tricholoma, Atrosquamosa,* and *Terrea* (Lukić & al., unpublished results).

Basidiomes were photographed in their natural habitat, at the area of their appearance. Morphological data were recorded from the fresh specimens, which were collected within a period of two months, on three occasions. Color codes follow the Munsell soil color charts (Munsell 1975). Two collections (about ten specimens in each collection) were dried by natural ventilation in the shadow and were preserved in sealed glass containers. Microscopic characters were observed under a Motic SFC 28 microscope (Germany), measured using an ocular micrometer, and recorded by a BCAM3 camera (Germany). The fresh sections of basidiomes were stained by Congo red. Basidiospore dimensions were recorded without 5% of the largest and 5% of the smallest measured values and Q indicates the length/width ratio of the spores. Measurements of the other microscopic characters are given as ranges. The voucher specimens have been deposited in the Fungarium at BUNS Herbaria, Department of Biology and Ecology, University of Novi Sad, Novi Sad, Serbia (BUNS).

Molecular & phylogenetic analysis

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). Amplification of ITS and LSU regions was performed under following conditions: 2 min denaturation at 95°C, followed by 35 amplification cycles, each consisting of 30 s denaturation at 95°C, a 30 s annealing step at 54°C, and 1 min extension at 72°C; the final step included 10 min incubation at 72°C. The primers ITS1F and ITS4B (White & al. 1990, Gardes & Bruns 1993) were employed toamplify the ITS rDNA region, while LR0R and LR5 (Gardes & Bruns 1993, Vilgalys

& Hester 1990) were used for the 28S rDNA region. PCR products were checked in 1% agarose gels, and amplicons weresequenced with one or both PCR primers. Sequence chromatograms were inspected and manually edited with FinchTV 1.5.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). Newly generated ITS and LSU sequences were deposited in GenBank.

Additional sequences were retrieved from GenBank or UNITE database: for ITS, 26 *Tricholoma* sequences from 15 taxa, with a *Clitocybe* outgroup sequence; and for LSU, 19 *Tricholoma* sequences from 13 taxa, with a *Ganoderma* outgroup sequence. A separate phylogenetic analysis was performed on each of the gene regions. Alignments for both analyses were carried out with MUSCLE 3.7 (Edgar 2004) using the default settings. Substitution models were selected using the ModelTest-NG v0.1.6 (Darriba & al. 2020). GTR + I + G (General time reversible model + Proportion of invariant + Gamma) model was chosen for ITS datasets while K80+I+G (Kimura 2-parameter model + Proportion of invariant + Gamma) was chosen for LSU dataset. Maximum Likelihood (ML) analyses were performed in MEGA X (Kumar & al. 2018) with the number of bootstraps set to 1000. MUSCLE 3.7andModelTest-NG v0.1.6 were used through CIPRES Science Gateway (Miller & al. 2010). The alignments and phylogenetic trees were deposited in Treebase.

Taxonomy

Tricholoma punctatum Lukić, sp. nov.
Figs 1–3

MB 842908

Basidiomes of *T. punctatum* differ by the presence of dark colored, elevated, circularly arranged spots on the lobed gray pileus.

Type: Serbia, Šumadija, Kragujevac, "Memorial complex of Sumarice", 240 m a.s.l., on soil in mixed thermophilous deciduous forest (*Quercus robur*, *Q. cerris*, *Carpinus betulus*), October 2020, Nebojša Lukić (**Holotype,** BUNS 12-00766; GenBank OK631797, OL310859).

Etymology: The specific epithet refers to the distinctly spotted surface of pilei.

Pileus: 50–110 mm, at first convex, becoming flattened with broad, low umbo. Fully developed pileus is often irregularly circular, lobed, with wavy margin (Fig. 1). Surface is densely felt, then breaking up in small fine scales. Pileipellis can be separated easily by half of the pileus radius. Color is gray [N7/0-N4/0], grey-brown [5YR6/4-5YR7/3] with lighter margin zone (Fig. 1 A–C). it is characterized by punctate, almost black spots (more or less pronounced) placed in one, usually two or even three circular zones at half and at 3/4 of the pileus radius (Fig. 1 A, C, D). These punctate black-colored spots are often elevated and can be removed by nail quite easily. Lamellae strongly sinuate (clearly expressed, Fig. 1 B, C), rather broad, quite thick and deep, whitish [N9/0], then pale grey [N7/0], not spotted. Intermediate lamellae numerous. The number of
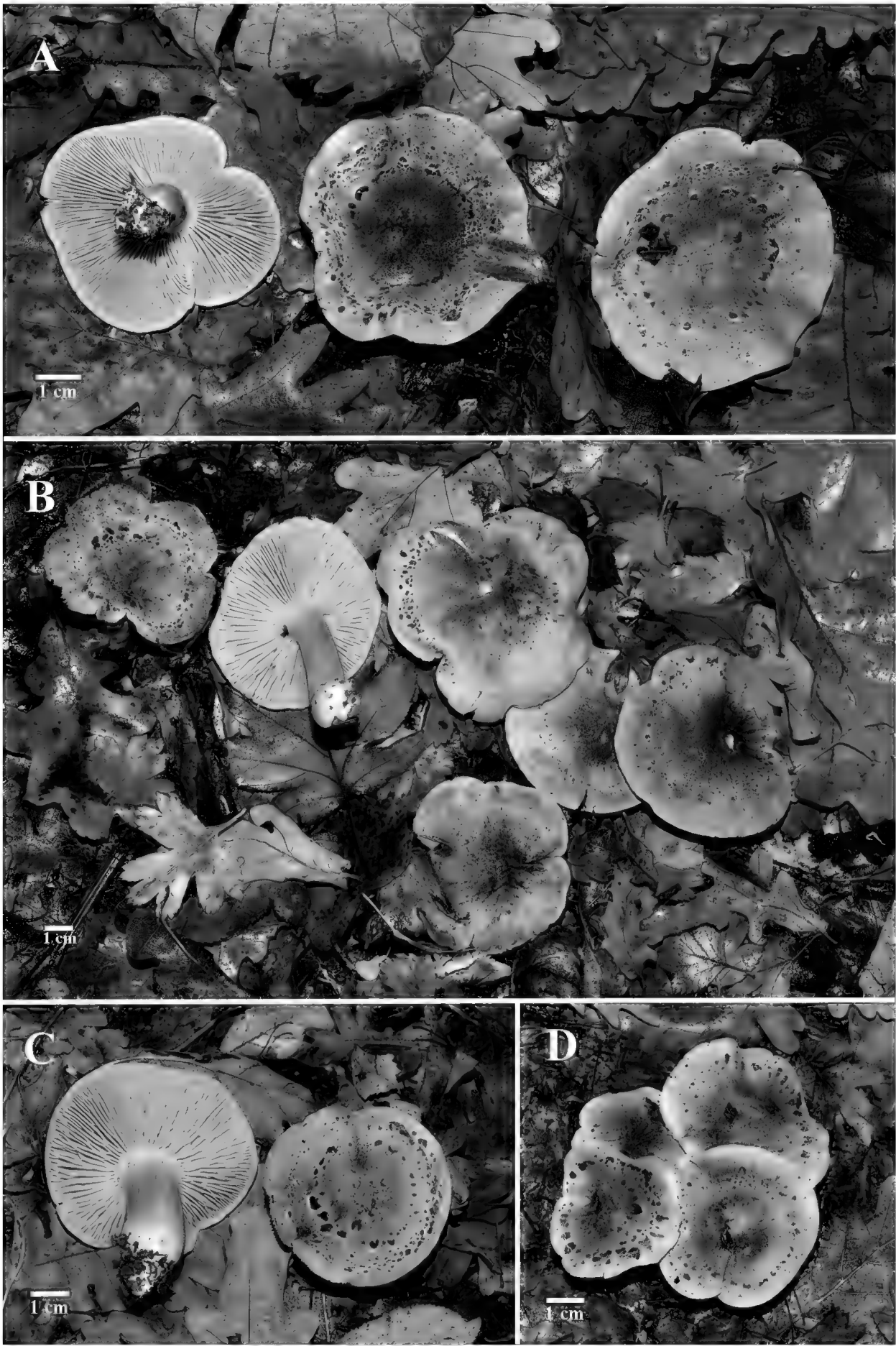
Fɪɢ. 1. *Tricholoma punctatum*: basidiocarps in situ.

those half-length lamellae is nearly equal to the number of ordinary lamellae. Edges are entire and wavy. Color changes are not observed. STIPE: 40–90 × 10–20 mm, robust and firm, cylindrical but not so rare flattened or with vertical grooves, widened at apex, tapering at base, white on ends, rough and covered for the most part with gray [N6/0] or gray-brown fibers (FIG. 1, B, C). No cobweb ring zone observed. Basal mycelium whitish. FLESH: white then faintly gray. ODOR: clearly farinaceous, strong after cutting. TASTE: farinaceous without bitter component.

BASIDIOSPORES: 4.6–5.1 × 3.6–4.1 µm (60 spores, from 3 basidiomes), subglobose to ellipsoid, Q = 1.20–1.35 (FIG. 2 A). BASIDIA: 25–35 × 3.5–6µm, mainly 4-spored (FIG. 2 B). PILEIPELLIS: a cutis, breaking upon trichoderm scales (FIG. 2 C); individual hyphal elements generally 33–75 × 7–14µm; subpellis poorly differentiated; mainly globose inflated cells 20-30 µm in diameter (fully developed), present as separated clusters in punctate colored elevated fibers within pileipellis (FIGS 2 and 3). CLAMP-CONNECTIONS: absent.
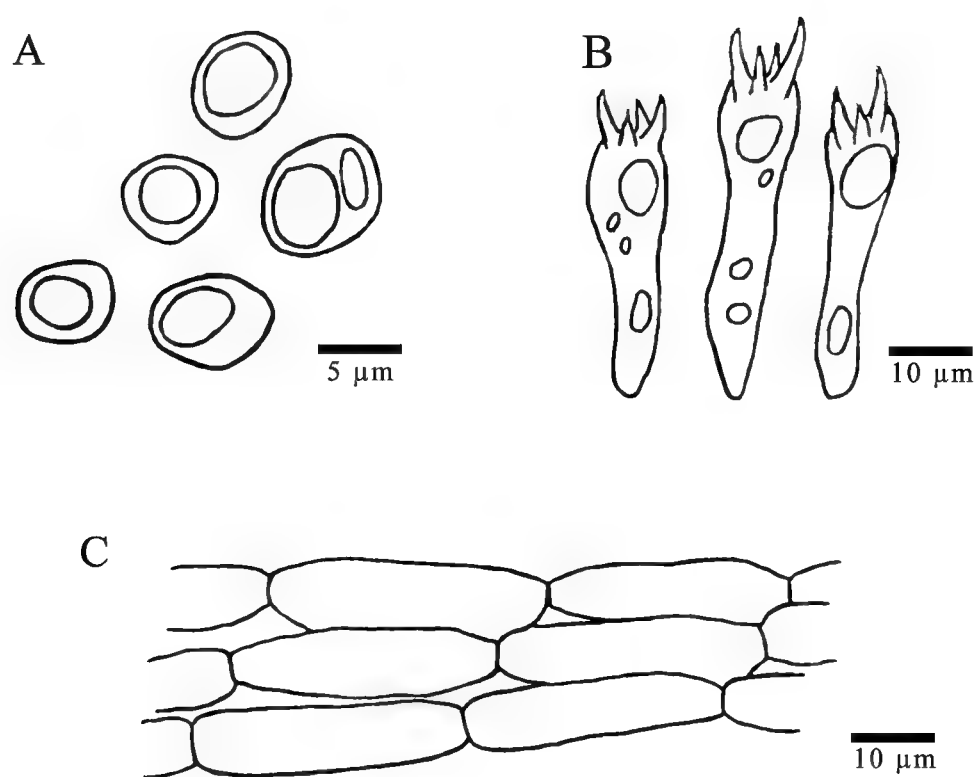

FIG. 2. *Tricholoma punctatum* (holotype BUNS 12-00766): A. basidiospores; B. basidia; C. pileipellis.

Fig. 3. *Tricholoma punctatum* (holotype BUNS 12-00766): globose inflated cells in punctate colored areas within pileipellis.

ADDITIONAL SPECIMEN EXAMINED: **SERBIA, ŠUMADIJA, Kragujevac**, "Memorial complex of Sumarice", 240 m a.s.l., on soil in mixed thermophilous deciduous forest (*Quercus robur, Q. cerris, Carpinus betulus*), October 2020, Nebojša Lukić (BUNS 12-00782, GenBank ON598607)

HABITAT & ECOLOGY: Basidiomes solitary or often in small groups. Habitat is a mixed thermophilous deciduous forest (*Quercus robur, Q. cerris, Carpinus betulus*) overgrown with *Crataegus monogyna, Prunus spinosa*, and *P. cerasifera*, on neutral or slightly acidic clayey soil.

Key to *Tricholoma* sect. *Atrosquamosa* Kühner & Romagn.

Cap greyish to almost black but also greyish-brown, densely squamulose-fibrillose, stem often squamulose too, smell different but farinaceous after cutting, spores medium sized.

1. Stem white, more or less shiny, often with weakly expressed, scattered blackish scales, smell of uncut fruit bodies sweetish 2
1. Stem white to greyish, with a dense covering of darker fibrils (or scales), smell of uncut fruit bodies spicy or farinaceous 3
2. Basal mycelium yellow, cap mostly fibrillose, especially gills and cap margin reddening when old, in deciduous forests, mostly *Fagus* and *Quercus* .... ***T. orirubens***
2. Basal mycelium white; cap mostly with dark (black) scales, with conifers, but also with *Tilia* and *Corylus* .... ***T. atrosquamosum***

3. In *Picea* forests (Fennoscandia), cap often with an olivaceous tinge, especially at margin .... *T. olivaceotinctum*

3 In thermophilous deciduous forests on clayey or calcareous soil, cap without olivaceous tinge 4

4. Stem less fibrillose or squamulose, widened stem base strongly reddening .... *T. basirubens*

4.Stem base not reddening 5

5. Cap margin woolly in young fruit bodies, cap strongly squamulose .... *T. squarrulosum*

5 Cap with conspicuous darker spots mainly arranged in two circular zones and often lobed, cap margin paler, spores mostly subglobose .... *T. punctatum*

Phylogenetic results

Newly generated ITS and LSU sequences of *Tricholoma punctatum* were approximately 780 bp and 750 bp long, respectively. BLAST sequence analysis showed maximum similarity with *T. orirubens* AMB 17410 (MT462641) for ITS sequence, while LSU sequence of *T. punctatum* showed maximum sequence match with *T. sulphurescens* G0243 (MK278617) and *T. orirubens* (MK278611) with exactly the same scores. Phylogenetic analysis of ITS (Fig. 4) and LSU (Fig. 5) datasets shows that ITS and LSU sequences of *T. punctatum* generated in this study formed their own lineage within the clade corresponding to sect. *Atrosquamosa*. In the analysis of ITS sequences *T. punctatum* was shown as sister clade of *T. orirubens*, while in the LSU tree *T. punctatum* is placed within the *T. orirubens* clade.

Discussion

Morphologically, the main distinguishing feature of *Tricholoma punctatum* is the pileus appearance and the presence of the globose inflated cells in punctate black-colored are as in pileipellis (Fig. 1). These characters are similar to those of *Tricholoma rufenum* P. Donati which possesses drop-like spots on the pileus. However, the pileus of *T. rufenum* is smooth, shiny and slightly greasy (similar to *T. portentosum* (Fr.) Quél.) (Riva 1998). In addition, *T. rufenum* has bigger spores (5.5–7.2 × 4.6–5.2 μm; Riva 2003) than *T. punctatum* and a white stipe. The drop-like spots are as smooth as the whole pileus surface, in contrast to the elevated spots of *T. punctatum*. Similar smooth drop-like spots appear on the pileus of *T. viridilutescens* M.M. Moser native to Europe. Similar pileus appearance can be also found in the *T. sculpturatum* var. *atrocinctum* Romagn. but the aforementioned feature is not constant (Heap 2020).
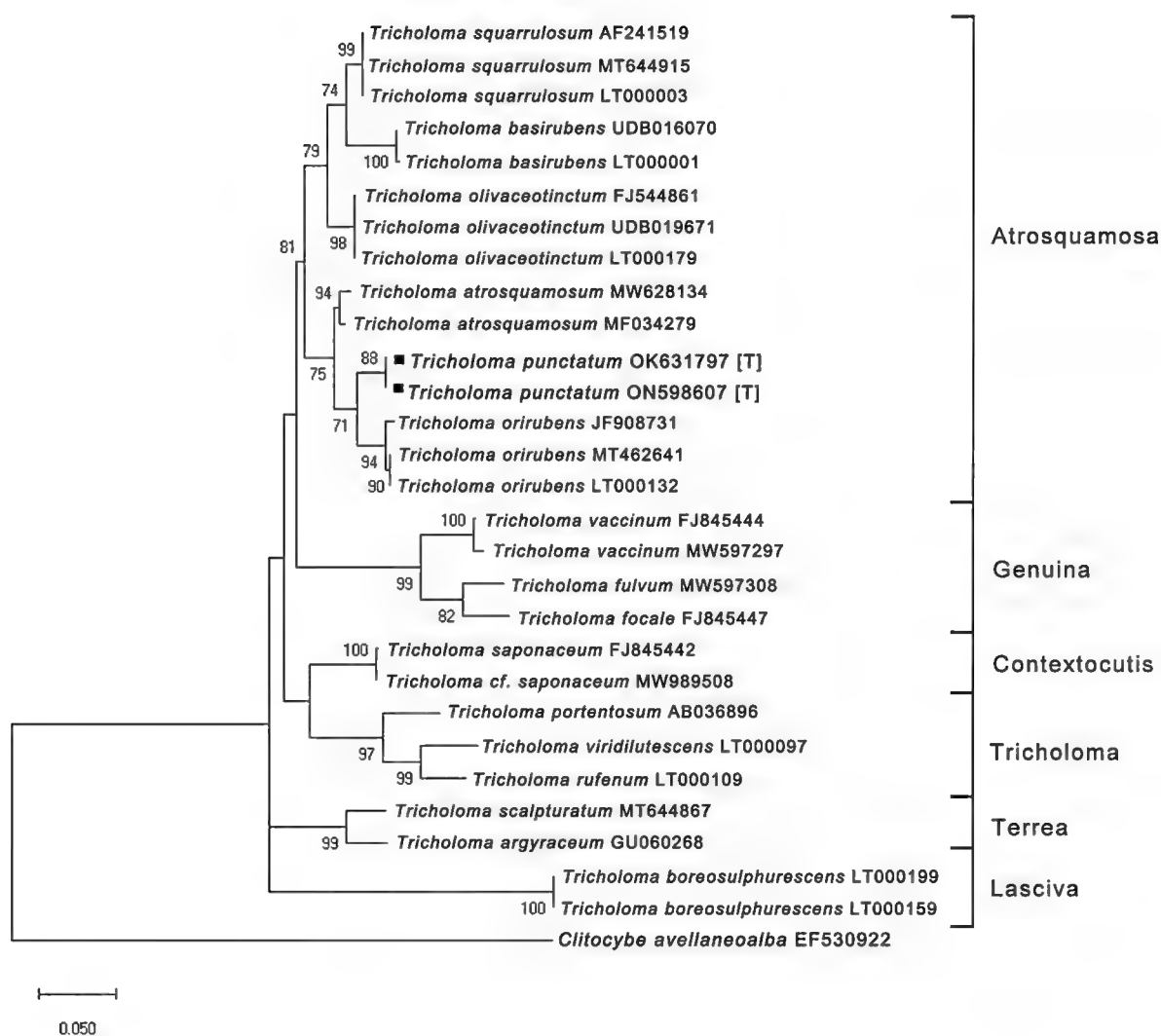
Fig. 4. Maximum likelihood tree inferred from the ITS sequences of *Tricholoma* species, with a *Clitocybe avellaneoalba* outgroup. Bootstrap values ≥50%, based on 1000 replicates are shown at the branch nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 28 *Tricholoma* nucleotide sequences. There were 914 positions in the final dataset.

Besides the particular pileus appearance, *T. punctatum* shows morphologically similar features with *T. orirubens* Quél., *T. atrosquamosum* Sacc., *T. squarrulosum* Bres. and *T. scalpturatum* (Fr.) Quél.

Unlike *T. punctatum*, *T. orirubens* has greenish yellow basal mycelium (Christensen & Heilmann-Clausen 2013), reddening lamellae (mainly in mature stage), white stipe and slightly bigger (and more elongated) spores. When compared with *T. punctatum*, *T. atrosquamosum* has dark gray to black pileus (no brown shade; Riva 1988), blackish spotted gills, white, shiny stem with blackish floccules or green (or red) spots at base, different smell of pepper (Baccardo & al. 2008), as well as distinct habitat and mycorrhizal partners and bigger and more elongated spores (Courtecuisse 1999). *Tricholoma atrosquamosum* is found in North America and Europe, but is generally
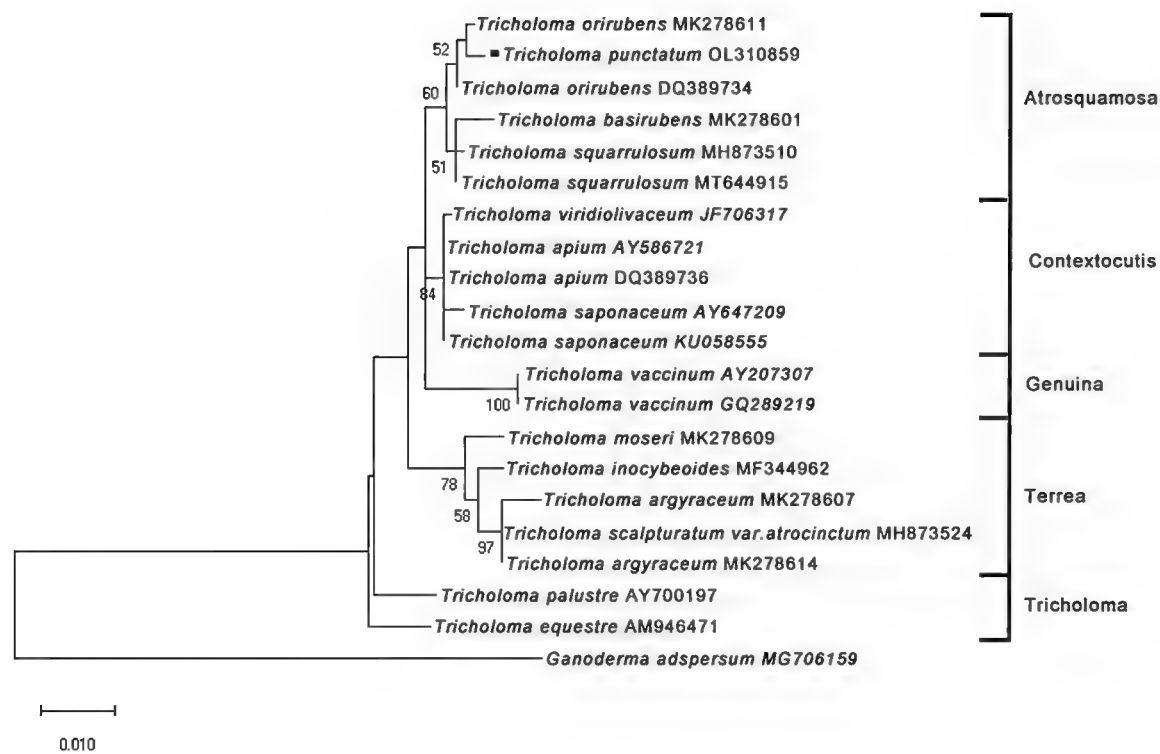
Fig. 5. Maximum likelihood tree inferred from the LSU sequences of *Tricholoma* taxa, with a *Ganoderma adspersum* outgroup. Bootstrap values ≥50%, based on 1000 replicates are shown at the branch nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 20 *Tricholoma* nucleotide sequences. There were 762 positions in the final dataset.

recognized as rare in Europe and in danger of extinction in the Netherlands (Noordeloos & Christensen 1999). Basidiomes appear under deciduous and coniferous trees, particularly beech and spruce on calcareous soils.

Tricholoma squarrulosum is smaller (Moser 1983, Phillips 2006), with dense black squamules on pileus with whitish woolly margin and strongly blackish squamulose stipe (Kibby 2010) while *T. punctatum* has grey fibrillose stem. *Tricholoma squarrulosum* also has different smell and bigger and more elongated spores (Q =1.35–1.72) (Christensen & Heilmann-Clausen 2013). *Tricholoma squarrulosum* is also rare (though more widely distributed in southern Europe), and associated with oak, pine, and spruce on chalk soils, with its basidiomes appearing from September to November.

Furthermore, comparing to *T. punctatum,* the species *T. scalpturatum* is smaller in size (Courtecuisse & Duhem 1995), with yellowing lamellae (Galli 2003), white or grey smooth stipe (sometimes with cobweb ring zone) and more elongated spores (Q = 1.4–1.54) (Christensen & Heilmann-Clausen 2013). Likewise, it should be emphasized that new species *T. punctatum* usually have lobed pileus and deep, strongly sinuate lamellae.

Beside the morphological differences between *T. punctatum* and related species, the former species showed differences on molecular level as well, which can be clearly seen from the phylogenetic tree obtained in analysis of ITS sequences (Fig. 4). *T. punctatum* sequence was grouped within the *Atrosquamosa* clade and placed as sister clade of *T. orirubens*. Contrary to this, in the LSU tree (Fig. 5) *T. punctatum* was placed within the *T. orirubens* clade. These results may be due to the fact that LSU is more conserved among species and have less clearly defined barcode gap than ITS (Schoch & al. 2012). Grouping of species in clades/sections in both analyses were in accordance with data of Heilmann-Clausen & al. (2017).

Conclusion

According to the data presented, *Tricholoma punctatum* belongs to section *Atrosquamosa*, which is one of ten proposed *Tricholoma* sections based on molecular and morphological data (Christensen & Heilmann-Clausen 2013). It is characterized by constant morphological features such are pileus color, pileipellis structure, absence of clamp connections and spores size and shape. This section is amongst those (sections *Tricholoma*, *Atrosquamosa*, *Pardinicutis*, and *Terrea*) which include species with a greyish, radially fibrillose, squamulose to felty pileus (Christensen & Heilmann-Clausen 2013). The main distinguishing characteristic of our new species is the presence of globose inflated cells in punctate areas of the pileus, and of the dark colored, elevated, circularly placed (in one to three tiers) spots on the cap. In addition, other characteristics that separate *T. punctatum* from similar species, *T. orirubens*, *T. atrosquamosum*, *T. squarrulosum*, and *T. scalpturatum*, are the size and shape of spores, fibrillose stipe, lobed pileus, strongly sinuate and deep gills. Furthermore, molecular data based on newly generated ITS sequences provided evidence supporting the distinct taxonomic status of *T. punctatum*.

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Acropleurophialis simplex gen. & sp. nov. from China

Ya-Fen Hu[1], Jing-Wen Liu[1], Xiu-Guo Zhang[2], Rafael F. Castañeda-Ruíz[3], Jian Ma[1*]

[1] *College of Agronomy, Jiangxi Agricultural University,*
   Nanchang, Jiangxi 330045, China
[2] *Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests,*
   College of Plant Protection, Shandong Agricultural University,
   Taian, Shandong 271018, China
[3] *Instituto de Investigaciones de Sanidad Vegetal,*
   Calle 110 No. 514 e/5ta B y 5ta F, Playa, La Habana 11600, Cuba
[*] Correspondence to: *majian821210@163.com; jxaumj@126.com*

Abstract—A new asexual ascomycete genus and species, *Acropleurophialis simplex*, collected on dead branches of an unidentified broadleaf tree in Jiangxi Province, China, is described and illustrated. The fungus is distinguished by macronematous, unbranched, determinate or percurrently extending conidiophores, and solitary, semi-endogenous, simple, smooth, spherical or subspherical, aseptate, pale brown to subhyaline conidia seceding schizolytically from polyphialidic, integrated, terminal and intercalary, sympodial, cylindrical conidiogenous cells with conspicuous collarettes.

Key words—hyphomycetes, saprobes, taxonomy

Introduction

Jiulianshan Mountain, located in the south of Jiangxi Province, China, lies at 24.4883–24.6486°N 114.3806–114.5256°E. It covers c. 134 km² and has a humid subtropical climate with an average annual temperature of 16.4°C and an average annual precipitation of 2155.6 mm. The main forest types are primary subtropical evergreen broadleaf forest. Such conditions are particularly suitable for the growth of microfungi. During our continuing survey of

saprobic hyphomycetes in this region, an interesting fungus was collected, that is significantly different from all previously described hyphomycetes (Seifert & al. 2011, Index Fungorum 2022), and therefore, a new genus, *Acropleurophialis*, is erected for it.

Materials & methods

Samples of dead branches were collected and placed in Ziploc™ plastic bags for transport to the laboratory, where they were processed and examined as described by Ma & al. (2011). Microphotographs were prepared using a Nikon Eclipse E200 and a SmartV550Dc digital camera, with a 100× (oil immersion) objective at the same background and scale. Adobe Photoshop 7.0 was used for image processing to assemble photographs into a plate. The studied specimens were deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, Jiangxi, China (HJAUP).

Taxonomy

Acropleurophialis Y.F. Hu, X.G. Zhang, R.F. Castañeda & Jian Ma, **gen. nov.**
IF 559774

> Differs from *Dictyochaeta* by its lack of setae, and its polyphialidic conidiogenous cells with spherical or subspherical, aseptate conidia; and from *Phialogeniculata* by its conidiogenous cells without stipitate collarettes, and its aseptate conidia.

> TYPE SPECIES: *Acropleurophialis simplex* Y.F. Hu & al.

> ETYMOLOGY: *Acro-* (Latin) meaning terminal + *pleuro-*(Latin) meaning lateral + *phialis* (Latin) referring to the phialidic conidiogenesis of this anamorphic fungus.

Asexual fungi. COLONIES effuse, brown to dark brown, hairy. Mycelium superficial or immersed. Setae and hyphopodia absent. CONIDIOPHORES macronematous, mononematous, unbranched, septate, smooth, determinate or indeterminate, with several enteroblastic percurrent extensions. CONIDIOGENOUS CELLS polyphialidic, integrated, terminal and intercalary, sympodial, cylindrical, with conspicuous collarettes. Conidial secession schizolytic. CONIDIA solitary, semi-endogenous, simple, smooth, spherical or subspherical, aseptate, pale brown to subhyaline.

Acropleurophialis simplex Y.F. Hu, X.G. Zhang, R.F. Castañeda & Jian Ma,
sp. nov.   FIG. 1
IF 559775

> Differs from *Dictyochaeta* spp. by its lack of setae, and its polyphialidic conidiogenous cells with spherical or subspherical, aseptate conidia; and from *Phialogeniculata* spp. by

its conidiogenous cells without stipitate collarettes, and its aseptate conidia.

TYPE: China, Jiangxi Province: Jiulianshan Mountain, on dead branches of an unidentified broadleaf tree, 2 Nov. 2013, J. Ma (**Holotype**, HJAUP M0083-2).
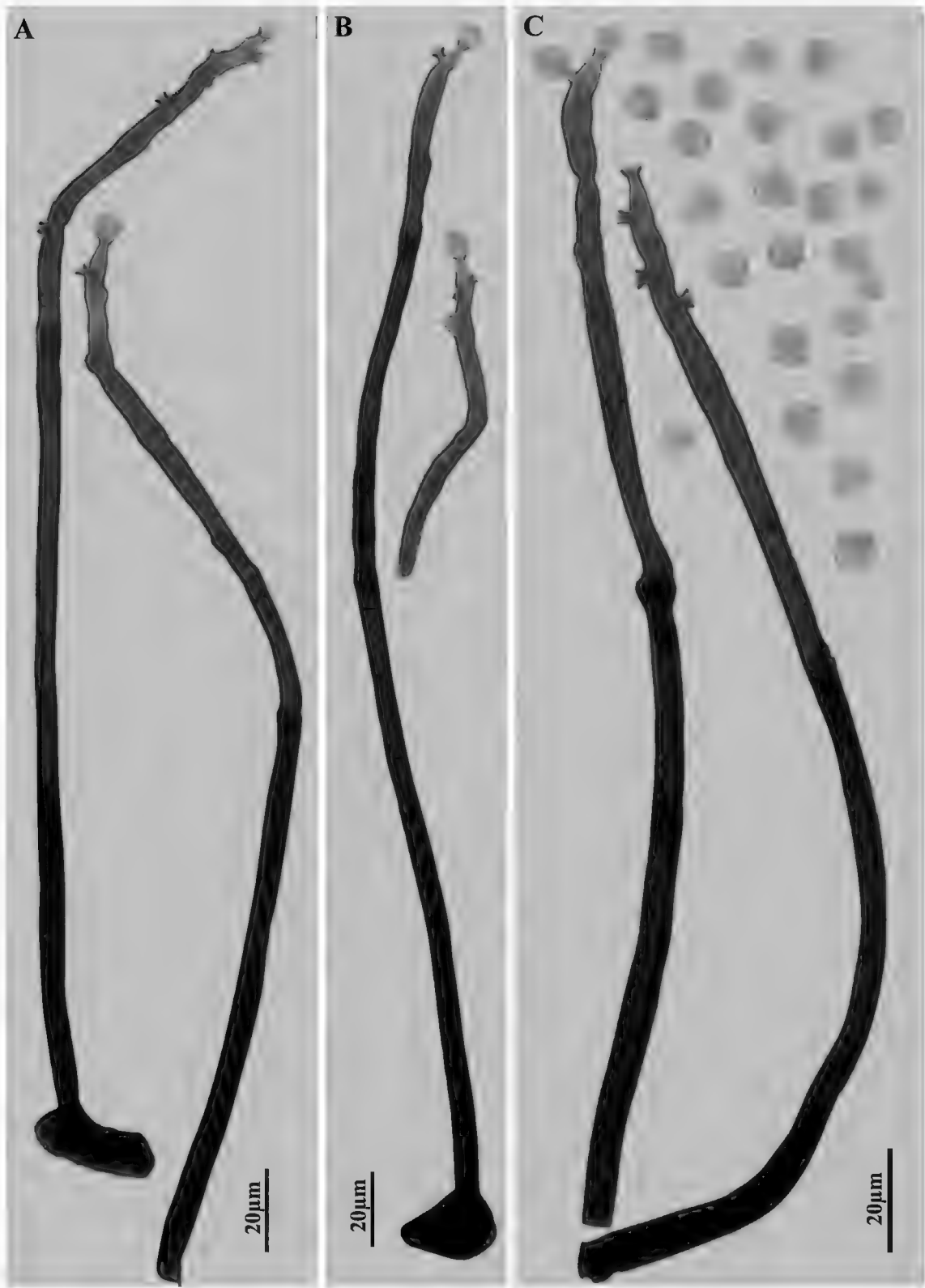
ETYMOLOGY: refers to the simple conidia.

FIG. 1. *Acropleurophialis simplex* (holotype, HJAUP M0083-2). Conidiophores, conidiogenous cells, and conidia.

Asexual fungi. Colonies on the natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial and immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae. Setae and hyphopodia absent. Conidiophores macronematous, mononematous, unbranched, 4–17-septate, smooth, brown to dark brown, paler towards the apex, determinate or indeterminate, with 1–2 enteroblastic percurrent extensions, 175–335 μm long, 5–7 μm wide. Conidiogenous cells polyphialidic, integrated, terminal and intercalary, sympodial, cylindrical, brown to pale brown, smooth, with conspicuous and flared collarettes, 19.5–36 × 5–6.5 μm. Conidial secession schizolytic. Conidia solitary, semi-endogenous, simple, smooth, spherical or subspherical, aseptate, pale brown to subhyaline, 7.5–8.5 μm in diameter.

Discussion

Acropleurophialis simplex is distinguished by its macronematous, unbranched, determinate or percurrently extending conidiophores with polyphialidic, integrated, terminal and intercalary, sympodial, cylindrical conidiogenous cells with conspicuous collarettes, and solitary, semi-endogenous, simple, smooth, spherical or subspherical, aseptate, pale brown to subhyaline conidia. It has similar conidial ontogeny to *Phaeoacremonium* W. Gams & al. (Crous & al. 1996), *Phialogeniculata* Matsush. (Kobayasi 1971), and *Dictyochaeta* Speg. (Spegazzini 1923). However, *Phaeoacremonium* differs by its aculeate conidiogenous cells and inconspicuous collarettes; *Phialogeniculata* differs by its geniculate conidiophores with several lateral apertures surrounded by brown, stipitate, funnel-shaped collarettes, and obclavate, septate conidia. In *Dictyochaeta*, the setae are frequently present beside conidiophores, and, together with the conidiophores, setae form small clusters, and mono- or polyphialidic conidiogenous cells produce typically falcate, 0–3-septate, slimy conidia, usually with a setula at each end (Gamundi & al. 1977, Kuthubutheen & Nawawi 1991, Whitton & al. 2000).

Several other genera, including *Phialophora* Medlar, *Craspedodidymum* Hol.-Jech., *Zakatoshia* B. Sutton, *Stephembruneria* R.F. Castañeda, *Anacraspedodidymum* C.R. Silva & al., and *Ramiphialis* F.R. Barbosa & al. have phialidic conidiogenesis like *Acropleurophialis* (Medlar 1915, Holubová-Jechová 1972, Sutton 1973, Castañeda-Ruíz 1988, Silva & al. 2014, Barbosa & al. 2020). However, the conidiogenous cells are monophialidic in these six genera, whereas those of *Acropleurophialis* are polyphialidic.

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MYCOTAXON

Trimmatothelopsis ireneana & T. wendyana spp. nov. from South Korea, with a key to Trimmatothelopsis

JUNG SHIN PARK[1], JOSEF P. HALDA[2], SOON-OK OH[1]*

[1] *Division of Forest Biodiversity, Korea National Arboretum, Pocheon 11186, South Korea*
[2] *Department of Biology, University of Hradec Králové, Hradec Králové, Czech Republic*
* CORRESPONDENCE TO: okkass15@korea.kr

ABSTRACT—*Trimmatothelopsis ireneana* and *T. wendyana* are described as new species based on collections from South Korea. *Trimmatothelopsis ireneana* is characterized by dark brown, punctiform apothecia having a raised dark brown apex, by the 0.1–0.3 mm wide, yellowish thallus areoles, and by a low (130–175 μm) hymenium. *Trimmatothelopsis wendyana* is characterized by reddish punctiform apothecia, by the smooth, yellowish brown, thallus areoles with round to irregular shape, overlapping as if a squamulose thallus, and by a high hymenium (220–250 μm). A phylogenetic analysis demonstrated the two species to be closely related and separated from other *Trimmatothelopsis* species as new clades in the mtSSU analysis. In the ITS analysis, the clade of the two new species was found to be a sister to *T. rhizobola*. A key is presented to all 13 accepted species of *Trimmatothelopsis*.

KEY WORDS—*Acarosporaceae*, taxonomy, *Acarospora*, lichens

Introduction

The lichen genus *Trimmatothelopsis* Zschacke belongs to the lichen family *Acarosporaceae*. It is morphologically similar to *Verrucaria* Scop., but it differs with multi-spored asci and persistent interascal filaments (Ertz & Diederich 2004). However the ascomata are apothecia with paraphyses rather than perithecia with paraphysoids (Gueidan & al. 2014, Knudsen & Lendemer 2016). Thirteen species have been described in the genus *Trimmatothelopsis*. They are distributed worldwide, and occur on rocks and soil (Gueidan & al. 2014, Knudsen & Lendemer 2016, Knudsen & al. 2011, Kondratyuk &

al. 2015, McCarthy 2008, Navarro-Rosinés & al. 1999, Roux & Navarro-Rosinés 2002). The largest number of species was recognized by Knudsen & Lendemer (2016), who transferred *Acarospora dispersa*, *A. rhizobola*, and *A. terricola*; *Melanophloea americana*, *M. coreana*, and *M. montana*; and *Thelocarpella gordensis* into *Trimmatothelopsis*, based on mitochondrial small subunit rDNA (mtSSU) analysis (Knudsen & al. 2016). In their molecular study, *Trimmatothelopsis* formed a strongly supported monophyletic clade that was sister to the clade with *Acarospora*, *Polysporina*, and *Sarcogyne*. An additional, three *Acarospora* species *(A. oreophila, A. sphaerosperma*, and *A. benedarensis*) have been transferred to *Trimmatothelopsis* based on molecular and micromorphological analyses (Knudsen & al. 2021).

The presence of *Trimmatothelopsis* in Korea has previously been overlooked. Using a molecular phylogenetic analysis, 54 of 124 *Acarospora* or *Endocarpon* samples appeared to belong to *Trimmatothelopsis*, including two unknown clades which are presented here as new species. These samples were identified preliminarily as *Acarospora* and *Endocarpon* spp. following traditional, morphology-based taxonomy, since they had apothecial ascomata similar to perithecial ascomata and brown thallus areoles. Seventeen species of Acarosporaceae (*Acarospora fuscata, A. hospitans, A. insolata, A. nitrophila, A. privigna, A. smaragdula, A. ulleungdoensis, A. veronensis, A. versicolor, Caeruleum heppii, Myriospora rufescens, Polysporina golubkovae, P. simplex, Sarcogyne clavus, S. regularis, S. ulleungdoensis*, and *Melanophloea coreana* [≡ *Trimmatothelopsis coreana*]) have been reported in South Korea (Aptroot & Moon 2014, 2015; Joshi & al. 2011; Kim 1981; Knudsen & Lendemer 2016; Kondratyuk & al. 2013, 2015, 2016a,b, 2017, 2018; Zhang & al. 2012). Here we provide characteristics of the new species, detailed morphological descriptions, and an identification key to all accepted species of the genus *Trimmatothelopsis* worldwide.

Materials & methods

Morphological study

All vouchers used in this study are deposited in the Herbarium, Korea National Arboretum, Pocheon-si, South Korea (KH) and the Herbarium, Korean Lichen Research Institute, Suncheon National University, Suncheon-si, South Korea (KoLRI). Morphological observations were made using a dissecting microscope (Olympus SZX7, Tokyo, Japan). The micromorphology of the ascomata was observed using a compound microscope (Olympus CX22LED, Tokyo, Japan). Cross-sections were cut by hand using razor blades and mounted in water. Color reactions on sections

of the hymenium were conducted using Lugol's iodine after pretreatment with 10% aqueous KOH solution (K/I). To identify the internal substances of each lichen, K (5% potassium hydroxide), C (aqueous solution of calcium hypochlorite), and P (paraphenylene diamine) solutions were used to examine the color reaction (chemical color tests; Orange & al. 2001). Chemical identification was performed by thin layer chromatography (TLC; Orange & al. 2001; Elix 2014)

DNA extraction and PCR amplification

Air-dried lichen thalli with ascomata were ground using a Mini-BeadBeater-16 (3450 rpm, 115 V, 10 A, BioSpec Products). DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA). PCR amplification was conducted using AmpliTaq DNA polymerase in corresponding buffer conditions. The following primers were used for PCR amplification: mtSSU1 and mtSSU3R for mtSSU (Zoller & al. 1999); and ITS1F (Gardes & Bruns 1993) and ITS4 and ITS5 (Zoller & al. 1999) for ITS. The PCR thermal cycling parameters were: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 30 s, and finally at 72°C for 3 min. The amplified DNA was concentrated and purified using a PCR quick-spin Product Purification Kit (iNtRON Biotechnology, Inc. Seongnam City, Korea), and then sent to Microgen Cooperation (Seoul, Korea) for sequencing.

Sequence alignments and phylogenetic analysis.

Specimens and sequences used in the phylogenetic analyses are listed in TABLE 1. Separate contigs were assembled and edited using SeqMan (Lasergene v. 7.1, DNASTAR). Sequence alignments were performed using ClustalW 1.83 (Thompson & al. 1994) We used *Trimmatothelopsis* sequences downloaded from GenBank and newly generated sequences: ITS sequences from 16 samples (*T. coreana* 8, *T. ireneana* 5, and *T. wendyana* 3) and mtSSU from 16 samples (*T. coreana* 7, *T. ireneana* 7, and *T. wendyana* 2). The outgroup sequences are the same as in previous publications (Knudsen & Lendemer 2016). Evolutionary analyses were conducted using MEGA X and MrBayes v. 3.2.6. Bayesian inference was performed on the data set using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes v. 3.2.6 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Best fit substitution models were estimated using Akaike information as implemented in jModelTest v 2.15 (Darriba & al. 2012). The F81 model was selected for mtSSU and ITS. Each MCMCMC run was performed with four independent Markov chains and 10 million generations. Trees were generated 1000 times, and the first 25% were discarded. The remaining trees were used for calculating a majority-rule consensus tree with posterior probabilities (PP). We also conducted maximum likelihood (ML) analyses using MEGA X with bootstrap value (BP), and selected the GTR+G model. Phylogenetic trees were constructed using Figtree v. 1.4.4 (Rambaut 2012) with TreeView X v. 0.5.0.

TABLE 1. Specimens and sequences of *Trimmatothelopsis* and *Pycnora* used in the phylogenetic analyses.

SPECIES	LOCALITY & VOUCHER	ITS	mtSSU
T. americana	USA, Lendemer 35758 (NY 1664)	—	KX578716
	USA, Lendemer 35758 (NY 1663)	—	KX578715
T. coreana	**South Korea, KH-L0007927**	**MT984219**	—
	South Korea, KH-L0007953	—	**MW001365**
	South Korea, KH-L0007959	—	**MW001366**
	South Korea, KH-L0007960	—	**MW001367**
	South Korea, KH-L0007961	**MT984217**	—
	South Korea, KH-L0012318	**MT984228**	**MW001368**
	South Korea, KH-L0012319	**MT984229**	**MW001369**
	South Korea, KH-L0012321	—	**MW001370**
	South Korea, KH-L0012322	**MT984230**	—
	South Korea, KH-L0005899	**MT984216**	—
	South Korea, KH-L0007967	**MT984215**	—
	South Korea, KH-L0007931	**MT984218**	**MW001364**
T. dispersa	USA, Lendemer 44571	—	KX578714
T. gordensis	France, CR22826	KM879337	KM879331
	France, CR25858	KM879338	KM879332
T. ireneana	**South Korea, KH-L0007356**	**MT984224**	**MW001355**
	South Korea, KH-L0007359	**MT984225**	**MW001356**
	South Korea, KH-L0007386	—	**MW001357**
	South Korea, KH-L0007401	**MT984227**	—
	South Korea, KH-L0007403	—	**MW001358**
	South Korea, KH-L0007404	—	**MW001359**
	South Korea, KH-L0007410	**MT984226**	**MW001360**
	South Korea, KH-L0007422	**MT984223**	**MW001361**
T. rhizobola	Sweden, Westberg 2994	EU870640	EU870692

Species	Locality & voucher	ITS	mtSSU
	Sweden, Westberg 3099	EU870641	EU870693
T. terricola	Czech, Knudsen 11216 & Sagar (S F256013)	LN810807	LN810932
	Czech, Knudsen 11216 & Sagar (S F256012)	LN810806	LN810931
T. versipellis	France, CR25921	KM879336	KM879327
	France, CR25922	KM879335	KM879328
T. wendyana	**South Korea, KH-L0007787**	**MT984221**	—
	South Korea, KH-L0002550	**MT984222**	—
	South Korea, KoLRI-010298	—	**MW001362**
	South Korea, KH-L0004196	**MT984220**	**MW001363**
P. sorophora	Sweden, Hermansson 7903a (UPS)	FJ959357	—
P. xanthococca	Sweden, Hermansson 11849 (UPS)	—	AY853339

Bold font indicates new sequences published in this study.

Results & discussion

The two phylogenetic analyses led to different tree topologies; the most likely trees from the ML analysis of ITS (Fig. 1) and mtSSU (Fig. 2) are presented here. The ITS region contained 568 bp, of which 366 bp were conserved and 200 were variable. The mtSSU region showed 684 bp, of which 595 bp were conserved and 89 were variable.

In the mtSSU tree, the Korean samples showed up in three separate clades, corresponding to *T. coreana* and two new species, here proposed as *T. ireneana* and *T. wendyana*. This is consistent with the results of previous studies that employed mtSSU (Gueidan & al. 2014, Knudsen & al. 2011, Westberg & al. 2015). Each species was highly supported in the phylogenetic tree. The new species *T. ireneana* and *T. wendyana* together form a clade with weak support (57/1). *T. coreana* formed a weak clade (57/1) with other European and American *Trimmatothelopsis* species. The ITS analysis (Fig. 1) showed a different relationship: the two new species formed a monophyletic clade with strong support values (98/1), and this clade together with *T. rhizobola* also had strong support (90/1). However, in the mtSSU analysis, the relationship between the two new species and *T. rhizobola* was not resolved, because of low support values. *Trimmatothelopsis coreana* formed a strongly supported
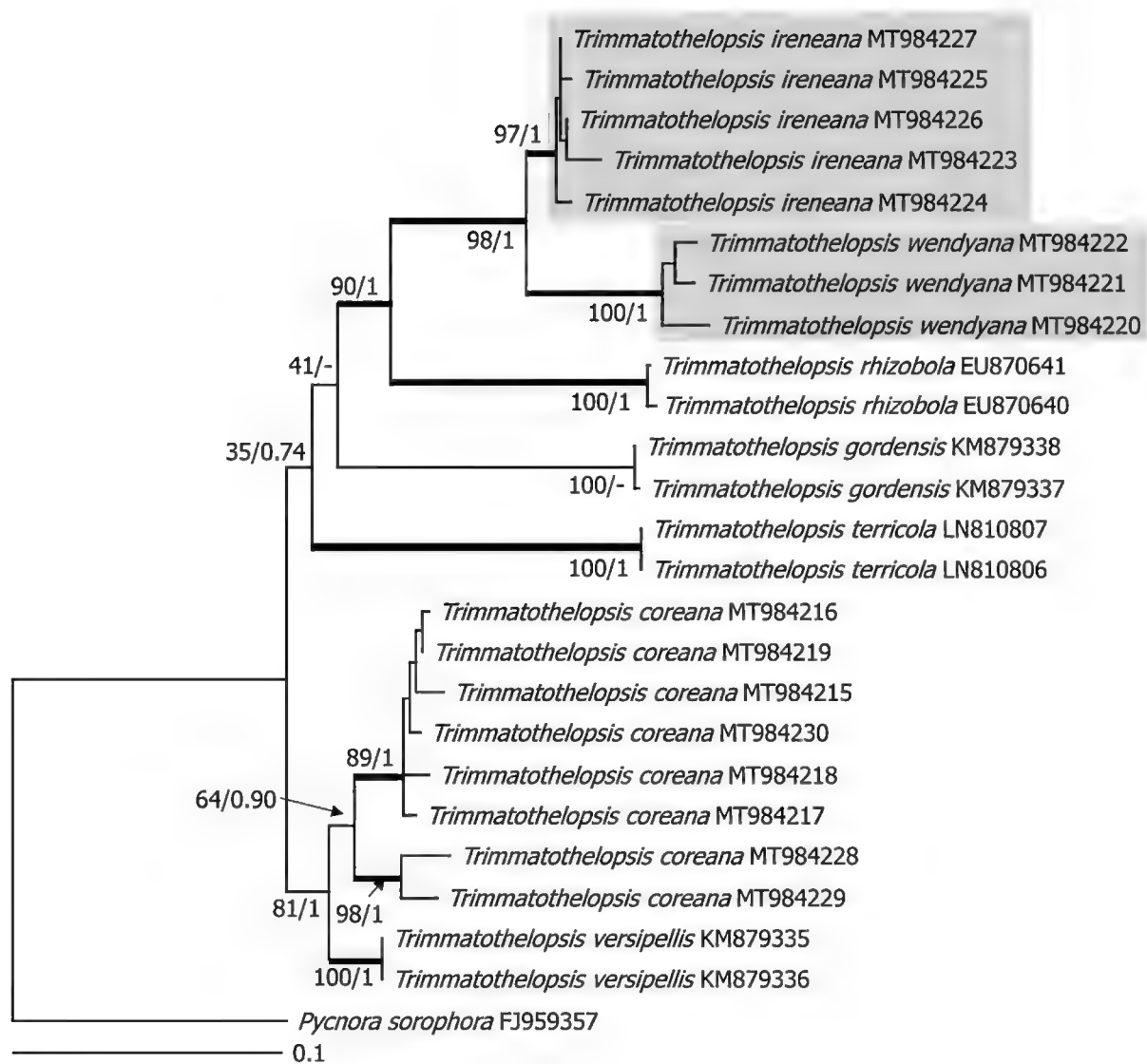
Fig. 1. Phylogenetic relationship in genus *Trimmatothelopsis*, based on Maximum Likelihood (ML) analysis of the internal transcribed spacer (ITS). Branches in bold indicate support of ML bootstrap ≥80% and Bayesian posterior probabilities ≥0.90. The clade shaded in grey corresponds with the two new species described in this study.

clade (94/1) and has a sister relationship with *T. americana, T. dispersa,* and *T. versipellis* (Fig. 2) with low support. Morphologically, the species is similar to *T. americana*; however, Knudsen & Lendemer (2016) examined the morphology of the *Trimmatothelopsis* species in order to identify morphological features that could serve as synapomorphies reflecting their shared evolutionary history. We applied their results to the two new species, and found that *T. ireneana* did not have pycnidia, unlike *T. dispersa* and *T. montana*, but has a high, globose hymenium with a disc size half or less of the equatorial diameter of the hymenium, and based on these morphological features this fits *Trimmatothelopsis*. *Trimmatothelopsis wendyana* also had a
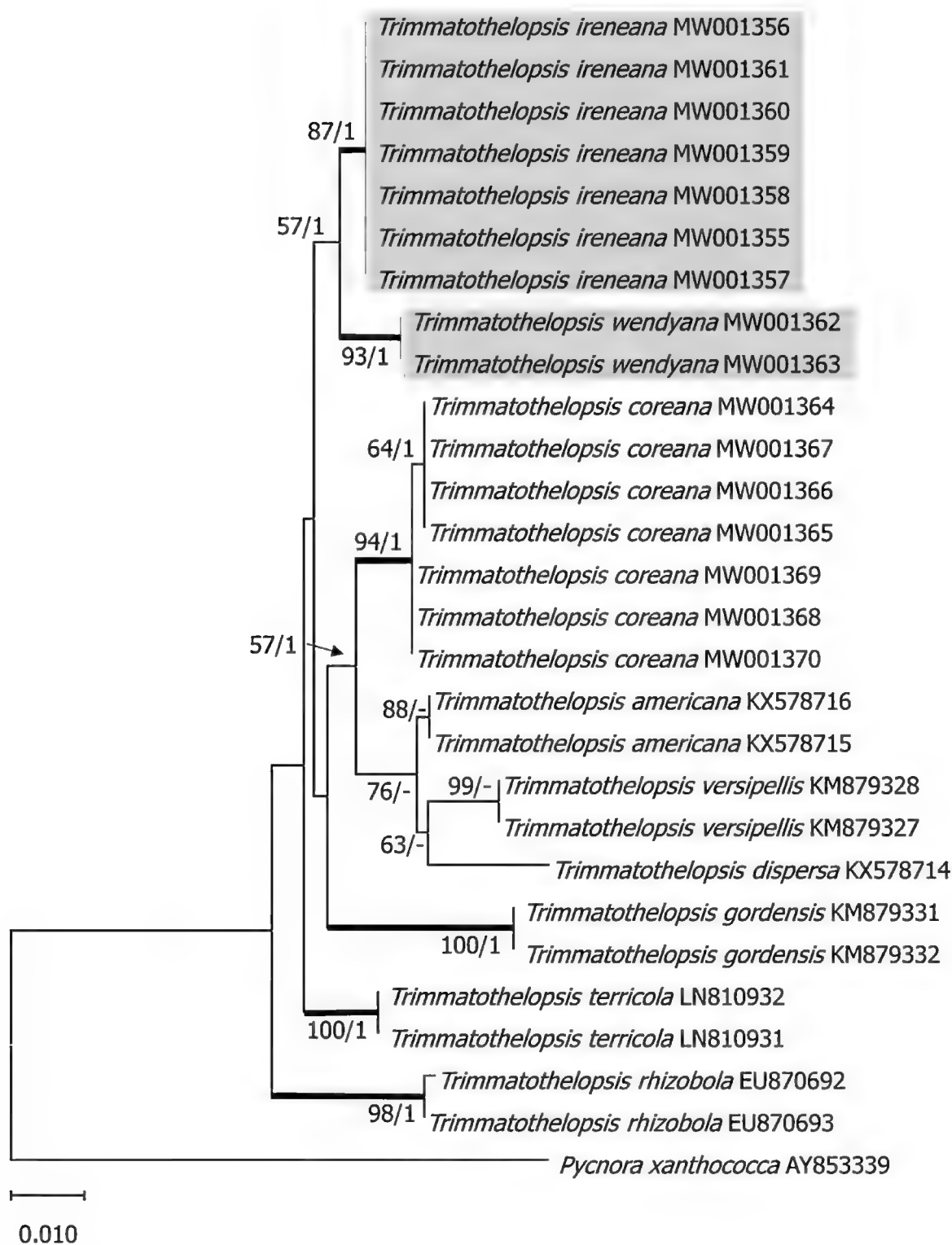
FIG. 2. Phylogenetic relationship in genus *Trimmatothelopsis*, based on Maximum Likelihood (ML) analysis of mitochondrial small-subunit (mtSSU). Branches in bold indicate support of ML bootstrap ≥80% and Bayesian posterior probabilities ≥0.90. The clade shaded in grey corresponds with the two new species described in this study.

high, globose hymenium, and long conidia (5–6 µm), and was equally included in *Trimmatothelopsis* because of morphological characteristics and molecular biological evidence. Most *Trimmatothelopsis* species have high hymenium (150–300 µm); but in four of the 11 recorded species it does not exceed 200 µm in height (*T. coreana, T. dispersa, T. terricola, T. versipellis*). *Trimmatothelopsis oreophila* has a high hymenium (170–220 µm) but a red-brownish thallus and thin areole size (0.1 mm vs. 0.5–1.5 mm). Further studies using additional genetic loci are required to confirm the significance of morphological features for recognizing the relationships between the species.

Taxonomy

Trimmatothelopsis ireneana J.S. Park & J. P. Halda, **sp. nov.**   Fig. 3
MB 837954

> Differs from *Trimmatothelopsis coreana* by its distinct, round to irregular thallus areoles, its small punctiform apothecia, its globose, low hymenium, and ithe absence of pycnidia.

> Type: South Korea, Jeju-do province, Seogwipo-si, Mt Halla, Yeongsil Trail, 33.3558°N 126.5003°E, alt. 1388 m, siliceous rock, 21 July 2015, J. Halda, K151652 (**Holotype**, KH-L0007404); K151605 (**Isotype**, KH-L0007395); K151651 (**Isotype**, KH-L0007403); K151653 (**Isotype**, KH-L0007405).

> Etymology: The species refers to Jeju-do, which is considered in Korea to be an island of peace. In Greek mythology, Irene is the name of the goddess of peace.

Thallus areolate, areoles distinct, dispersed or somewhat contiguous replicating by division, usually single and widely dispersed, 0.2–0.3(–0.8) mm wide, mostly plane, tightly attached. Upper surface yellowish brown to medium brown, epruinose. Epicortex absent. Eucortex with upper layer yellowish brown to dark brown and approximately 10 µm thick, paraplectenchymatous, and with a lower layer of hyaline, hyphal bundles penetrating the algal layer and 20–25 µm thick. Algal layer mixed with the lower cortex layer, 87.5–125 µm thick, uneven, and jagged in most areoles. Medulla white, prosoplectenchymatous, continuous, with attaching hyphae connected to the substrate, up to 150–180 µm thick, hyphae thin-walled and mostly 1 µm in diameter. Apothecia punctiform, diameter 0.1–0.3 mm, slightly concave in the middle of the discs, some of the apothecia having a raised dark brown apex, epruinose, mostly one per areole, and immersed in thalline warts, disc not carbonized; Parathecium paraplectenchymatous, hyaline, 12–15 µm thick; Epihymenium medium brown to blackish brown, color dissolving in KOH; Hymenium globose, hyaline, K/I–, 130–175 µm tall; Subhymenium up to 20 µm thick, hyaline with oil droplets; Paraphyses 1–2.5 µm at mid-level, not
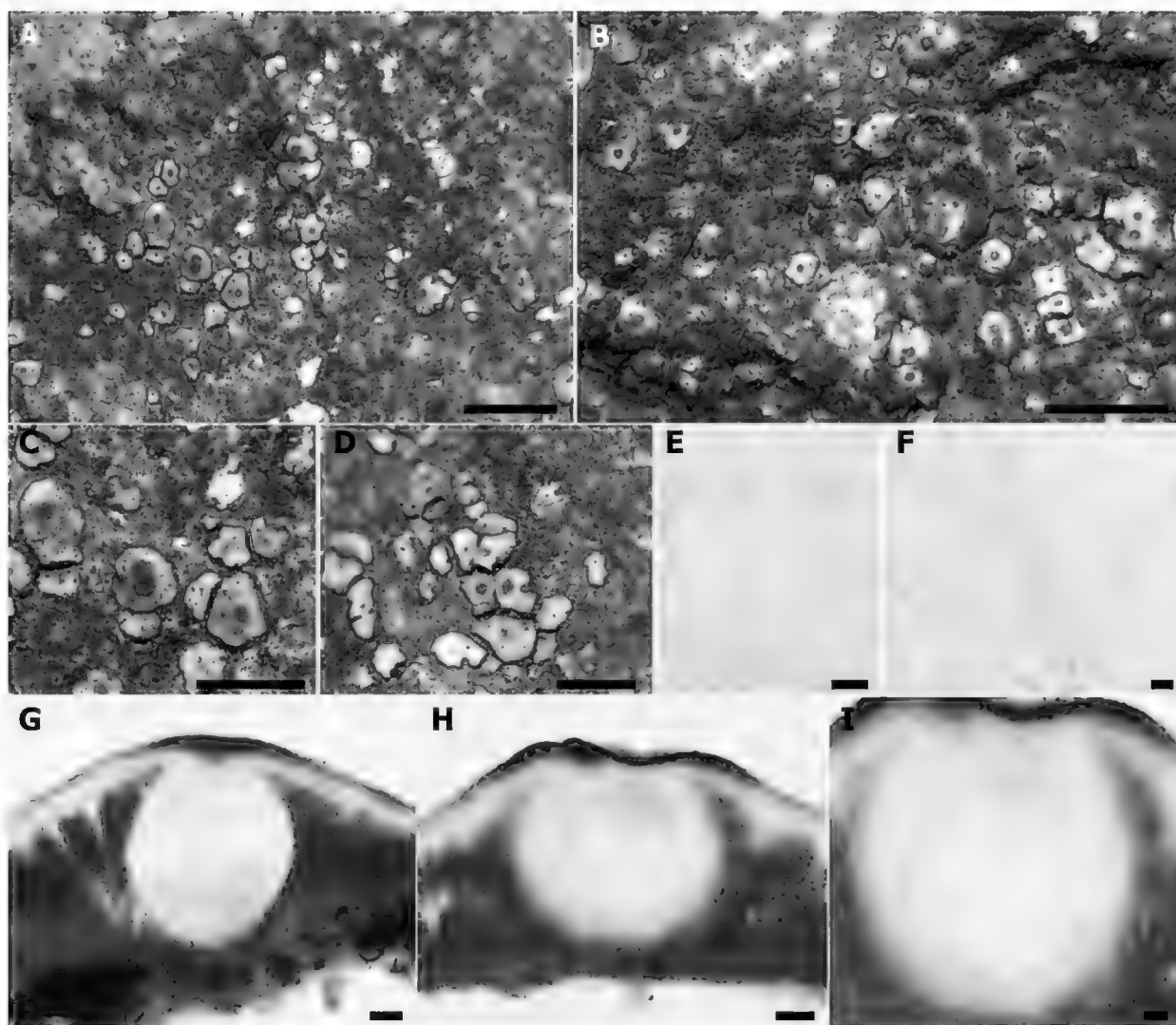
Fig. 3. *Trimmatothelopsis ireneana* (A–D = holotype, KH-L0007404; E–H = isotype, KH-L0007403; I = KH-L0007359): A. Thallus areoles with brownish punctiform apothecia; B. Continuous areoles with brownish punctiform apothecia; C. Close-up of apothecia having raised dark brown apex; D. Close-up of brownish punctiform apothecia; E. Paraphyses; F. Ascospores; G, H. Vertical section of an ascoma, uneven algal layer; I. Hymenium with ascus. Scale bars: A–D = 0.5 mm; E, F = 10 μm; G, H = 50 μm; I = 20 μm.

swollen at the apices. Asci narrowly ellipsoid, with K/I–, I– tholus, 130–135 × 20–30 μm, polysporous, containing up to 200–300 spores. Ascospores hyaline, simple, cylindrical, mostly 5 × (1–)1.5 μm. Pycnidia not observed.

Chemistry—No secondary metabolites detected.

Distribution and ecology—Collected only on Mt Halla on Jeju Island, where it grows on siliceous rocks at high altitudes in the mountain region. Known only from the type locality.

Remarks—This species is characterized by brownish punctiform apothecia, 0.1–0.3 μm wide, yellowish areoles, and a low (up to 175 μm in height) hymenium. Four species of *Trimmatothelopsis* share a negative iodine reaction in the hymenium: *T. coreana*, *T. dispersa*, *T. terricola*, and *T. wendyana*.

Trimmatothelopsis terricola differs from *T. ireneana*, because it grows on biological soil crusts and has hyphal bundles, while in growing on rocks and lacks hyphal bundles. *Trimmatothelopsis ireneana* is similar to *T. coreana* in the punctiform apothecia with a parathecial ring, but differs by its smooth, brown apothecia that are smaller (0.1–0.3 mm vs. 0.3–0.5 mm), and its distinct brownish thallus. The new species is also similar to *T. dispersa*, which differs in its K/I+ blue reaction, whereas *T. ireneana* has a negative K/I color reaction. *T. ireneana* differs from *T. wendyana* in apothecia morphology; *T. wendyana* is distinguished by a red-colored punctiform disc and high hymenium (220–250 µm vs. 130–175 µm).

> **_Trimmatothelopsis wendyana_** J.S. Park & S.O. Oh, **sp. nov.**   Fig. 4
> MB 843928
>
> Differs from *Trimmatothelopsis dispersa* by its distinct round and squamuloid thallus areoles, its taller hymenium, and its pycnidia with bacilliform conidia.
>
> Type: South Korea, Gangwon-do province, Yanyang-gun, Seo-Myeon, Peak of Galjeongokbng, 37.8691°N 128.4357°E, alt. 1101 m, siliceous rock, 22 May 2009, Y. Joshi & W.Y. Wang 090630 (**Holotype**, KoLRI-010298); Y. Joshi & W.Y. Wang 090638 (**Isotype**, KoLRI-010305).
>
> Etymology: This species is named in honor of Wendy, the favorite singer of the first author of this paper. Wendy had a serious accident that was very difficult to recover from, but after years of hard work she returned to the stage and is singing again.

Thallus areoles distinct, dispersed, widely spaced or contiguous, sometimes extensive and often forming a dense crust, areoles more or less squamule-like, solitary to overlapping, round to irregularly shaped with an entire margin, 0.3–1.2(–1.5) mm in diam., mostly plane but some of the areoles convex, rim ± down-turned, often with a distinct black margin. Upper surface dull, yellowish brown, epruinose. Epicortex thin or absent. Eucortex with upper layer yellowish brown to pale brown, 8–10 µm thick, paraplectenchymatous, lower layer hyaline, of hyphal bundles, 37.5–62.5 µm thick. Algal layer including the lower cortex layer 45–100 µm thick, uneven, interrupted by hyphal bundles. Medulla hyaline, prosoplectenchymatous, continuous, with attaching hyphae connected to substrate, 50–100 µm thick. Apothecia punctiform, diameter 0.1–0.3 mm, solitary or up to 1–2 per squamules; Disc usually reddish brown, immersed, smooth, epruinose, with reddish stain surrounding the disc on the upper surface, not carbonized; Epihymenium medium brown, the color dissolving in KOH, 20–25 µm thick; Hymenium globose, hyaline, K/I–, 220–250 µm tall; Subhymenium hyaline, up to 25–37.5 µm thick. Paraphyses 0.6–0.8 µm thick at mid-level, not swollen at the apices. Asci narrowly ellipsoid,
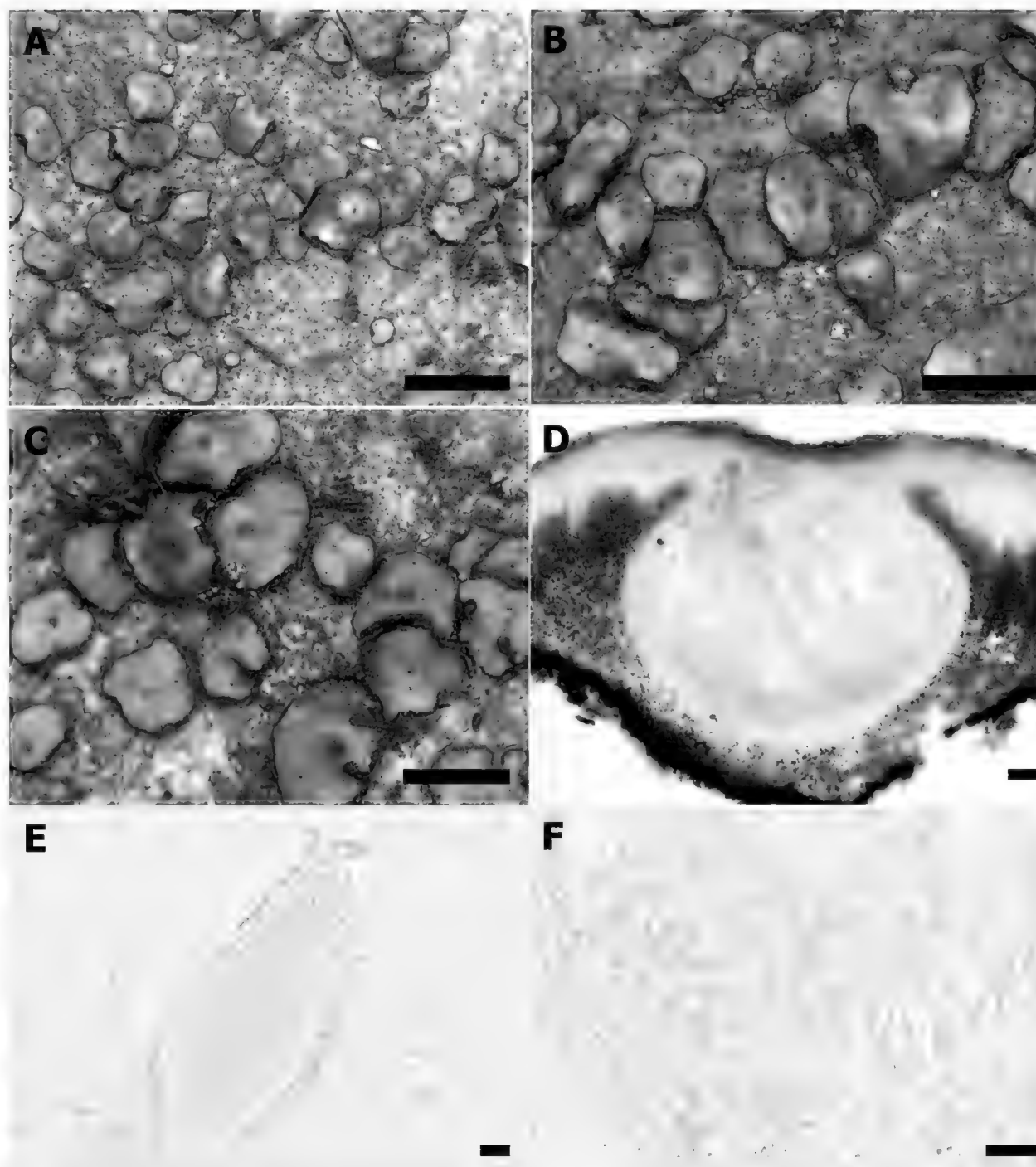
Fig. 4. *Trimmatothelopsis wendyana* (holotype, KoLRI-010298) A. Thallus areoles with developed apothecia; B. Close-up of reddish punctiform apothecia and squamule-like overlapping areoles; C. Close-up of reddish, punctiform, immersed apothecia; D. Vertical section of apothecia and hymenium; E. Ascus and ascospores; F. Conidia. Scale bars: A–C = 0.5 mm; D = 40 μm; E, F = 8 μm.

with K/I–, I– tholus, 100–110 × 13–15 μm, polysporous, containing up to 100–200 spores. Ascospores hyaline, simple, cylindrical, 4.7–5.3 × 1.3–2.1 μm. Pycnidia not numerous, resembling punctiform apothecia, 100–120 μm wide, up to 175 μm high, dacryoid to globose, conidia bacilliform, cylindrical, 5–6 × 1 μm.

Chemistry—No secondary metabolites detected.

DISTRIBUTION & ECOLOGY—It grows on siliceous rocks at mainly high altitudes in the mountain region.

ADDITIONAL SPECIMENS EXAMINED—**SOUTH KOREA, GYEONGSANGBUK-DO, Mungyeong-si,** Mungyeong-eup, Gjigok-ri, Mt Juheul, 36.7750°N 128.1081°E, alt. 1070 m, on rock, 29 February 2004, J. S. Hur, 040146 (KoLRI-000913); **Gyeongju-si,** Geeoncheion-eup, Mt. Danseok, 35.8178°N 129.0839°E, alt. 185 m, on rock, 16 June 2016, S.-O. Oh, C.S. Kim, Y.N. Kwag, S.K. Han KL16-0257 (KH-L0007787).

REMARKS—This species has a reddish to brownish punctiform apothecia and is mistaken for a species of *Endocarpon*. It is similar to *T. dispersa*, which has a reddish brown disc, lower hymenium size, with pycnidia not being observed, In contrast, *T. wendyana* has a distinct red punctiform disc, high hymenium (220–250 μm vs. 150–200 μm) with pycnidia and 5–6 μm bacilliform conidia. *T. wendyana* is similar to *T. ireneana* and *T. coreana*, but the latter two species can be distinguished by their distinct thalli. *T. ireneana* and *T. coreana* have small size areoles (0.3–0.5 mm) and are not overlapping, while *T. wendyana* has larger, overlapping areoles (1.5–2 mm). Moreover, it has a high hymenium (220–250 μm vs. 130–190 μm in *T. ireneana* and *T. coreana*).

Key to Trimmatothelopsis species (updated from Knudsen & Lendemer 2016)
1. Thallus on soil (terricolous) 2
1. Thallus on rock (saxicolous) 4
2. Squamules peltate, deep red to dull brown; on peaty soil over mica schist
.... *T. rhizobola*
2. Squamules crust-forming, yellowish or reddish brown to black, on soil of
coastal cliffs in sunny exposures 3
3. Squamules angular to irregular, 1–3 mm wide, apothecia inconspicuous
when dry, 1–5 per areole .... *T. benedarensis*
3. Squamules round to irregular, up to 1.3 mm wide, apothecia often lacking,
usually one per areole .... *T. terricola*
4. Ascospores mostly 0.5–1.5 μm wide, eastern North America 5
4. Ascospores mostly 2–2.5 μm wide, eastern North America or elsewhere 6
5. Thallus indistinct, apothecia discoid, arising individually, superficial,
broadly attached .... *T. americana*
5. Thallus distinct, squamulose, apothecia punctiform, 0.1–0.2 μm wide,
often sterile .... *T. oreophila*
6. Thallus on calcareous rock 7
6. Thallus on non-calcareous rock 9
7. Ascospores spherical, over 5 μm wide .... *T. sphaerosperma*
7. Ascospores ellipsoid, not over 5 μm wide 8
8. Tholus at least faintly amyloid; known only from Europe (coastal France)
.... *T. gordensis*

8. Tholus non-amyloid; widespread in North America .... *T. dispersa*

9. Hymenium K/I+ blue 10

9. Hymenium K/I– 11

10. Thallus areolate, round to irregularly shaped; apothecia punctiform,
solitary or up to six or eight in dividing areoles .... *T. dispersa*

10. Thallus not areolate, sparingly to richly rimose, smooth, apothecia resembling
perithecia, convex to hemispherical, known only from Australia .... *T. montana*

11. Apothecial disc carbonized; known only from Europe (France) .... *T. versipellis*

11. Apothecial disc not carbonized; known from Asia or North America 12

12. Thallus indistinct; apothecial disc round, forming a conspicuous, dark brown to
black apex on the apothecia, slightly raised, resembling perithecia .... *T. coreana*

12. Thallus distinct; apothecial disc punctiform, brown to reddish brown 13

13. Thallus continuous, with overlapping, squamule-like areoles, apothecia reddish;
hymenium mostly over 220–250 μm high .... *T. wendyana*

13. Thallus dispersed, without overlapping squamule-like areoles; apothecia dark
brown; hymenium 130–175 μm high .... *T. ireneana*

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MYCOTAXON

Steccherinum filiferum sp. nov. from the neotropics, and a new combination for *Odontia laxa*

Eugene Yurchenko[1*], Karl-Henrik Larsson[2,3], Janett Riebesehl[4], Otto Miettinen[5]

[1] *Institute of Forest Sciences, Białystok University of Technology,*
    Wiejska 45A, Białystok 15-351, Poland
[2] *Natural History Museum, University of Oslo,*
    Blindern, P.O. Box 1172, Oslo 0318, Norway
[3] *Gothenburg Global Biodiversity Centre, University of Gothenburg,*
    Box 461, Gothenburg SE-40530, Sweden
[4] *Institute for Plant Protection in Horticulture and Urban Green, Julius Kühn Institute,*
    Messeweg 11/12, Braunschweig DE-38104, Germany
[5] *Finnish Museum of Natural History Luomus, University of Helsinki,*
    P.O. Box 7, Helsinki FI-00014, Finland
* Correspondence to: *yauheni.yurchanka@pb.edu.pl*

Abstract—The new species *Steccherinum filiferum*, from Puerto Rico and Amazonian rainforests of Ecuador, is described. It is characterized by effused basidiomata with hyphal cords, a monomitic hyphal system, simple-septate hyphae, and minute hymenophoral aculei with encrusted, septate, *Candelabrochaete*-like cystidia. Because *Odontia laxa* [≡ *Odonticium laxum*] is morphologically and phylogenetically similar to *S. filiferum*, the new combination *Steccherinum laxum* is proposed, after studying its type. Scanning electron microscopy studies show that *S. filiferum* and *S. laxum* can be differentiated by crystal size and arrangement on the cystidia. A key to the *Steccherinum* species with simple-septate generative hyphae is provided.

Key words—Caribbean, palm leaves, *Polyporales*, septocystidia, *Steccherinaceae*

Introduction

Steccherinum Gray (*Steccherinaceae, Polyporales*) is a large genus with worldwide distribution. Ten species were listed for temperate northern hemisphere (Jülich & Stalpers 1980) and 27 species for tropics, subtropics, and the southern hemisphere (Hjortstam & Ryvarden 2007). Seventy-two species are accepted in this genus according to MycoBank (Robert & al. 2022). The species of the genus are saprobes on decaying wood, bark, and soft plant debris, and an ability for white rot is indicated (Ginns & Lefebvre 1993).

The morphological concept of this genus changed significantly over the last 50 years. According to an earlier concept (Maas Geesteranus 1974), *Steccherinum* species are characterized by spinose hymenophore, dimitic hyphal system, clamped generative hyphae, thick-walled tramal cystidia with crystalline incrustations, and variously shaped basidiomata from effused, effused-reflexed, to flabelliform and short-stipitate. In subsequent taxonomic works, three species with simple-septate generative hyphae (Svrček 1973, Ryvarden 1978, Hjortstam 1984, Liu & Dai 2021, Wu & al. 2021) and several species with poroid hymenophore (Knudsen & Hansen 1996, Miettinen & Ryvarden 2016) were included in this genus. As a consequence, the morphological distinctions among the genera *Irpex* Fr., *Steccherinum*, and *Junghuhnia* Corda were blurred. The polyphyletic nature of the two latter genera was confirmed by a multigene phylogenetic reconstruction (Miettinen & al. 2012). A further expansion of *Steccherinum* morphological concept resulted after studies by Liu & Dai (2021) who described *S. fragile* Z.B. Liu & Y.C. Dai, a species possessing a monomitic hyphal system, simple septa, poroid hymenophore, and lack of cystidia.

In this paper, a new species of *Steccherinum* is described from the neotropics, and *Odontia laxa* is transferred to *Steccherinum*.

Materials & methods

Specimens of the new *Steccherinum* were obtained during collection trips to Puerto Rico (K.H. Larsson, in 1996) and to South and Central America (E. Yurchenko, in 2019). In Ecuador, specimens were collected on dead wood along a route and in a sample plot 20×20 m in forests less influenced by human activities. Specimens examined are deposited in the Herbariums of Białystok University of Technology, Hajnówka, Poland (BLS) and University of Gothenburg, Göteborg, Sweden (GB).

Basidiome descriptions are based on dry specimens. Micromorphology was studied on basidiome sections mounted in 3% aqueous KOH solution. Crystalline incrustations and spores were examined in Melzer's reagent, and

cyanophily of spores was observed in 0.02% cotton blue in 50% aqueous lactic acid. A Nikon Eclipse Ni-U light microscope (Nikon Corp., Japan) was used for measurements, mostly under ×1000 magnification using the NIS-Elements Br imaging software (Nikon Corp.). Mean spore length (L) and width (W) were calculated as arithmetic averages for 30 randomly selected spores. Spore quotient, Q, is the length/width ratio for individual spores.

Scanning electron (SEM) images were obtained using Phenom G2 pro desktop microscope (Labmate, UK). Pieces of basidiomata, 3–4 mm in extent, were removed from dried specimens, attached by double-sided adhesive film to metallic stands, specific to this model of microscope, and coated with 1.4 nm layer of gold in a Leica EN ACE200 vacuum coater (Leica Microsystems, Germany).

DNA extraction was done with E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-Tek, VWR, Radnor, Pennsylvania, USA). Primers ITS1, ITS2, ITS3 (White & al. 1990) and ALR0 (Collopy & al. 2001) were used for DNA amplification of ribosomal internal transcribed spacer (ITS) region that included the ITS 1, ITS 2, and 5.8S gene. PCR products were purified with a DNA Clean & Concentrator®-5 kit (Zymo Research, Irvine, California, USA), then sequenced by LGC Genomics GmbH (Berlin, Germany). The ITS sequence was edited and assembled with MEGA X (Kumar & al. 2018) and the five quality check guidelines were applied (Nilsson & al. 2012). Sequence was deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank).

Results

The new ITS sequence OP279612 from a specimen from Ecuador (BLS M-5230) is 99.1% similar to JN710524 (*Candelabrochaete* sp., Miettinen & al. 2012), obtained from a specimen collected in Puerto Rico (KHL 9495, GB-0087369). We believe that the 0.9% difference in ITS sequences (with three mismatch positions and three positions with ambiguity symbols) indicate that they are the same species. The ITS sequence of *Odonticium laxum* JN710577, based on KHL 12268, had the next highest similarity (89.75%) with our new sequence in a BLAST search.

The phylogenetic analysis of Miettinen & al. (2012, Fig. 4) placed the new species as a sister taxon to *Odonticium laxum* KHL 12268 within the clade of *Steccherinum* s.str. To ascertain the identity of *O. laxum* KHL 12268, we compared it with an isotype specimen at GB. Both specimens have effused, minutely odontioid basidiomata (FIG. 1) with similar hyphae, hymenial elements, basidiospores, and crystalline incrustations on the cystidia (FIG. 2).
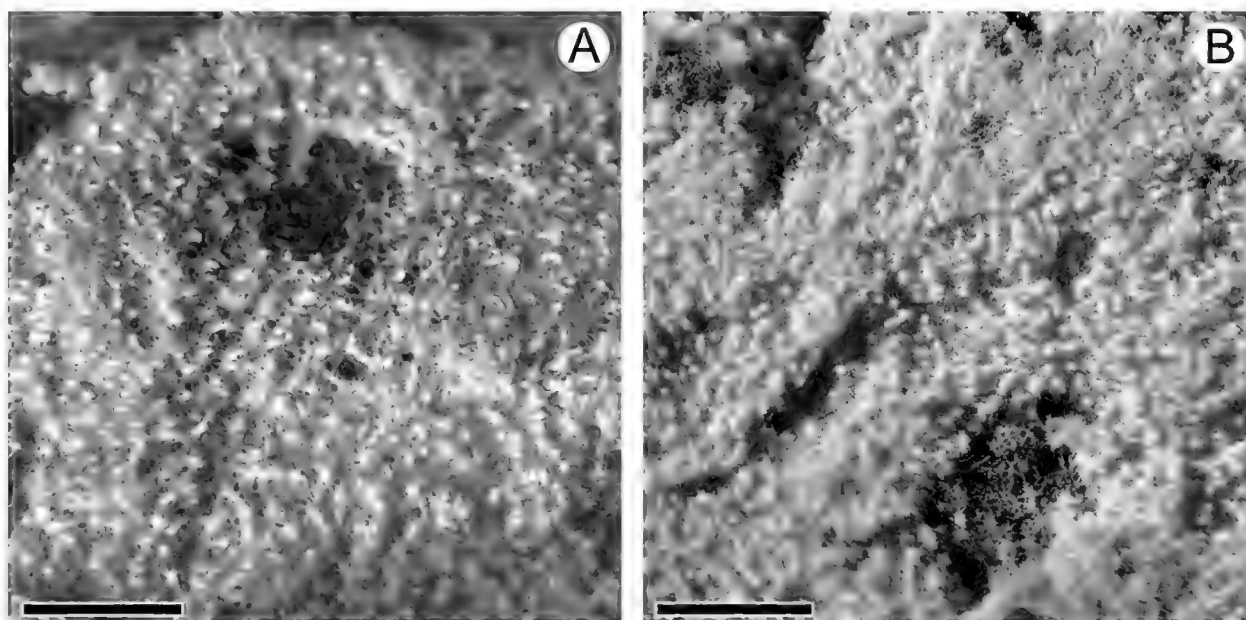
Fig. 1. *Steccherinum laxum*, macromorphology: A. GB KHL 12268; B. isotype of *Odontia laxa* (GB 19282). Scale bars = 1 mm.

Both specimens possess similar hyphal cords also. The basidiospores were similar in size and shape: L = 2.8 μm, W = 1.8 μm in KHL 12268; L = 2.9 μm, W = 1.9 μm in the isotype. Specimen KHL 12268 has a denser hyphal composition of aculei and 5–7 aculei/mm, compared to 6–8 aculei/mm in the isotype. Also, encrusted cystidia are barely visible or absent in some aculei in KHL 12268, whereas they are numerous and more distinctly encrusted in the isotype, resulting in a setose appearance of the aculei. From these observations, we conclude that KHL 12268 is conspecific with the isotype of *O. laxum*. Based on these phylogenetic and morphologic results, we propose the new combination *Steccherinum laxum* below.

The morphology of the new species was compared with the descriptions of all species of *Steccherinum*, *Candelabrochaete* Boidin, and *Odonticium* Parmasto, listed in Index Fungorum (http://www.indexfungorum.org/names/names.asp) with enough information for an adequate comparison. Because no published taxon was like our new species in micro- and macromorphology, and based on ITS sequence data, we propose a new species, described below.

The new taxon, *Steccherinum filiferum*, is morphologically similar or even identical with *S. laxum* in its hyphal, basidial, and spore morphology. However, we observed differences in crystalline incrustations on the cystidia in KOH solution and Melzer's reagent. SEM study revealed significant differences in the size and attachment of the crystals. In *S. laxum*, the crystals are primarily large, plate-like, and broadly attached (Fig. 3A), giving the cystidia a rough, thick-walled appearance at ×1000 magnification (Fig. 2D). In comparison, the
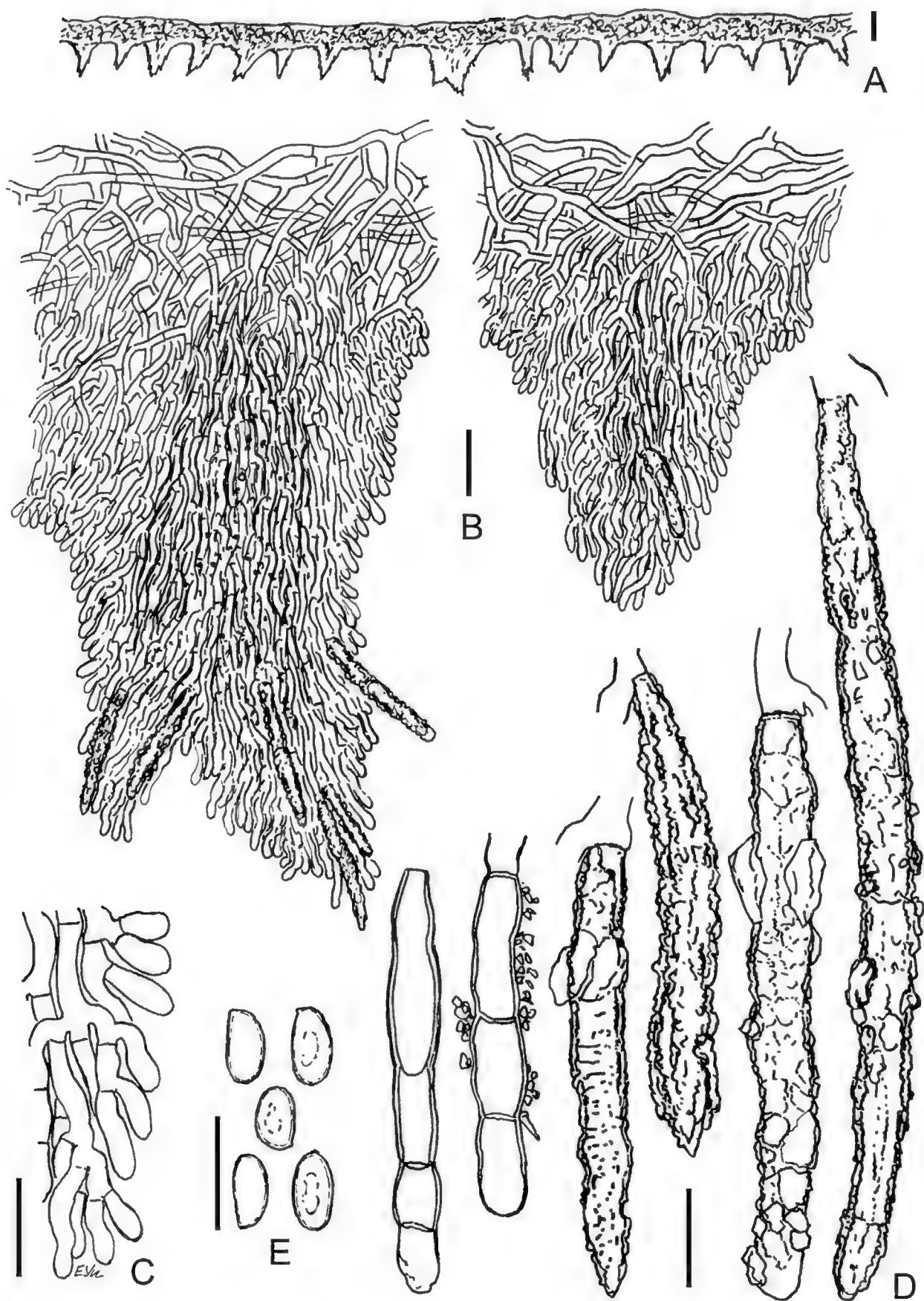
Fig. 2. *Steccherinum laxum* (GB KHL 12268): A, B. vertical sections through basidioma; C. portion of aculeal apex; D. smooth and variously encrusted cystidia; E. basidiospores. Scale bars: A = 100 μm; B = 20 μm; C, D = 10 μm; E = 5 μm.
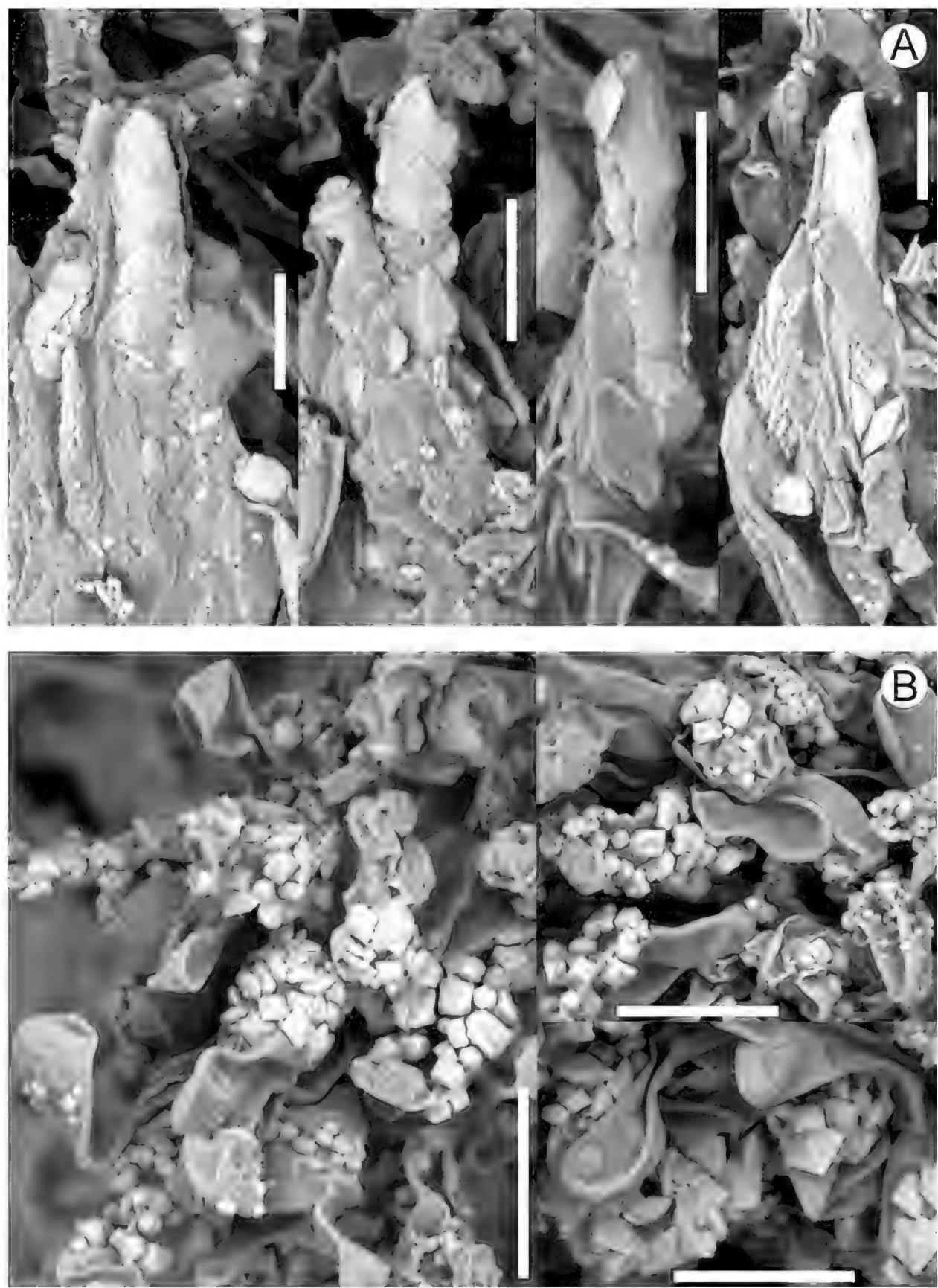
Fig. 3. SEM images of crystalline deposits on cystidia: A. *Steccherinum laxum* (isotype, GB 19282); B. *Steccherinum filiferum* (holotype, GB-0087369 [KHL 9495]). Scale bars = 10 μm.

crystals in the new *Steccherinum* are smaller, distinct, aggregated, and appear loosely attached (FIG. 3B).

Taxonomy

Steccherinum filiferum Yurchenko & K.H. Larss., **sp. nov.**   FIGS 3B, 4–6

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Differs from *S. laxum* by its cystidia encrusted with loosely attached aggregates of isodiametric crystals, and lack of numerous smooth hyphoid elements in upper part or aculei.

TYPE: USA, Puerto Rico, Isabela municipality, Bosque Estatal de Guajataca (Guajataca Commonwealth Forest), Montañas Aymamón, 18.4242N 66.9678W, 230 m a.s.l., moist subtropical forest, on decaying corticated branch of angiosperm tree on the ground, leg. K.-H. Larsson, 26.VI.1996, KHL9495 (**Holotype**, GB-0087369; GenBank JN710524, JN710613, JN710662, JN710713).

ETYMOLOGY: from the Latin *filiferum* = carrying threads, due to the presence of hyphal cords.
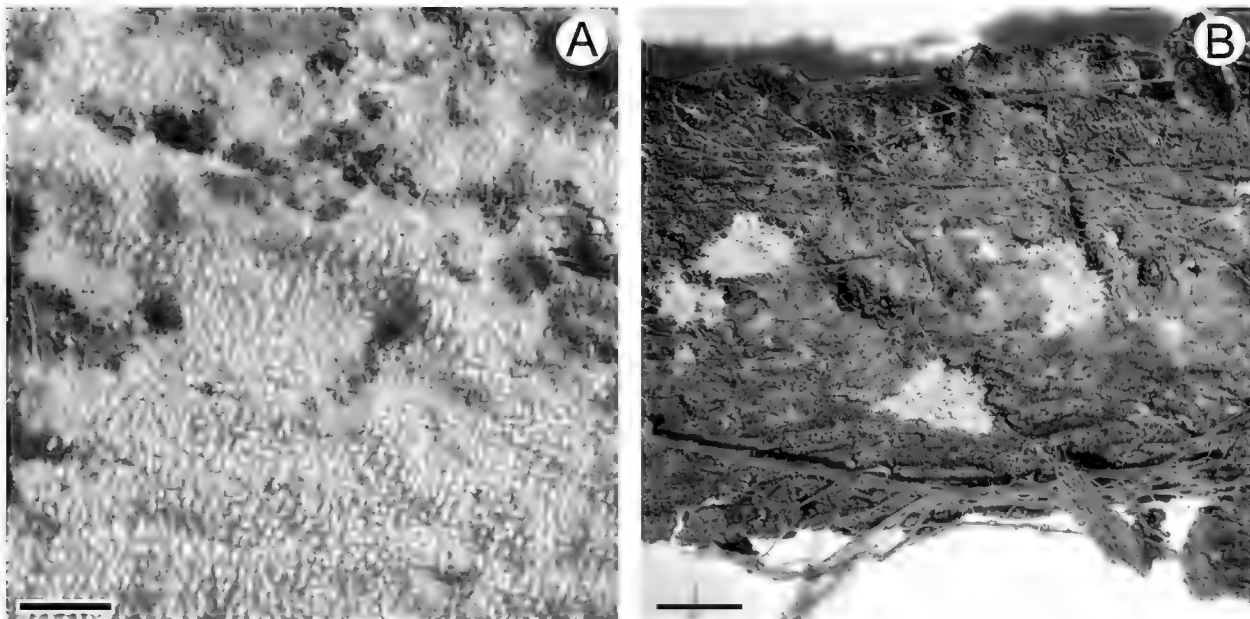

FIG. 4. *Steccherinum filiferum*, macromorphology: A. holotype, GB-0087369 (KHL 9495); B. BLS-M 5228, with basidiomata and hyphal cords. Scale bars = 1 mm.

BASIDIOMA effused, from 0.5–3 mm to 5 cm and more in extent, cream or pale ochraceous, soft-membranous and very fragile when young, membranous in older state, minutely odontoid, between aculei 25–100 μm thick, discontinuous to continuous or minutely cracked. ACULEI subcylindrical, blunt, 50–150 μm high, 30–80 μm in diam (5–8/mm). MARGIN abrupt or arachnoid and then concolorous with hymenial surface, 0.3–0.5 mm wide.
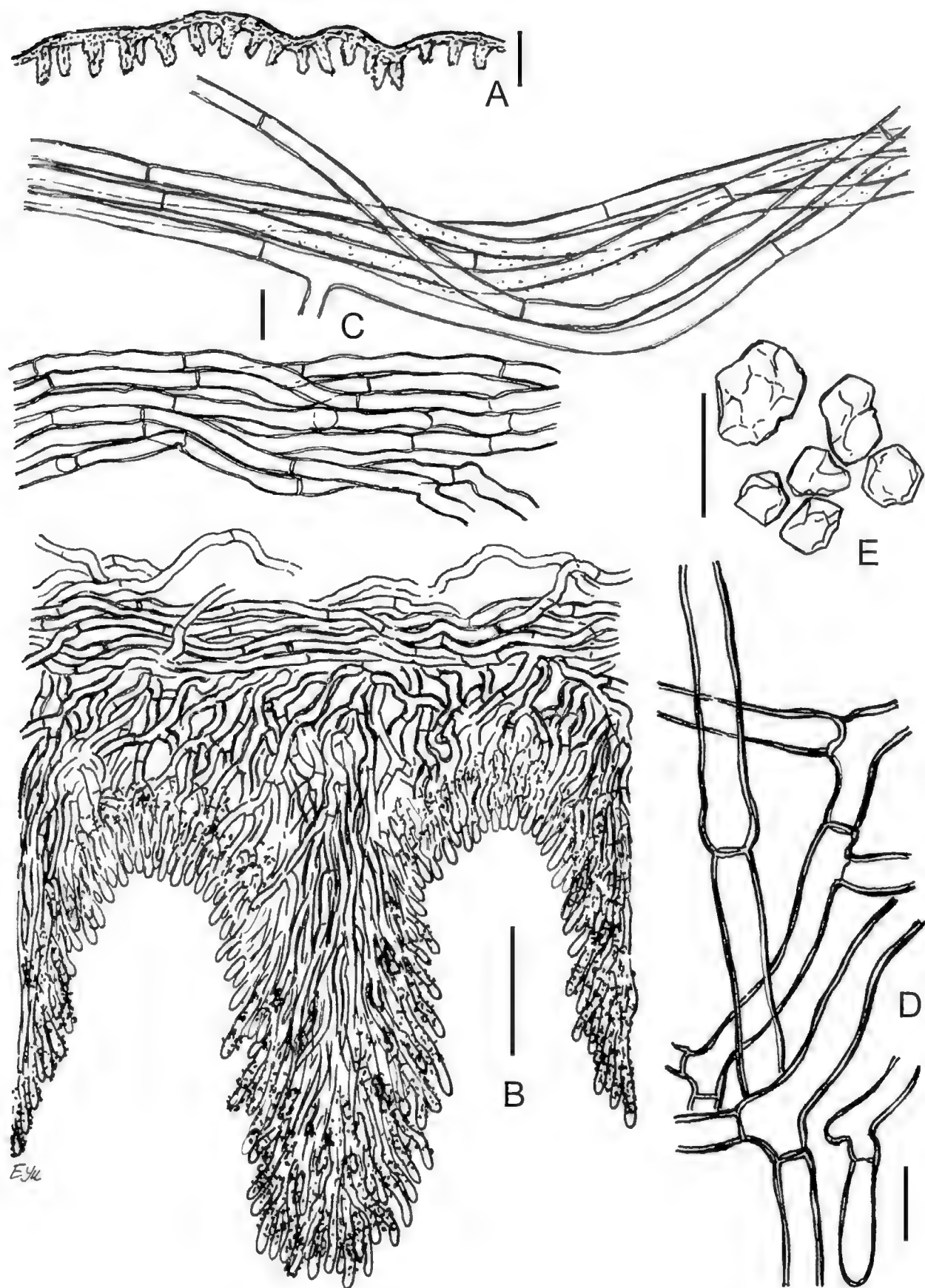
FIG. 5. *Steccherinum filiferum* (holotype, GB-0087369 [KHL 9495]): A, B. vertical sections through basidioma; C. hyphal cords; D. subicular hyphae; E. crystals from subiculum. Scale bars: A = 200 µm; B = 50 µm; C–E = 10 µm.
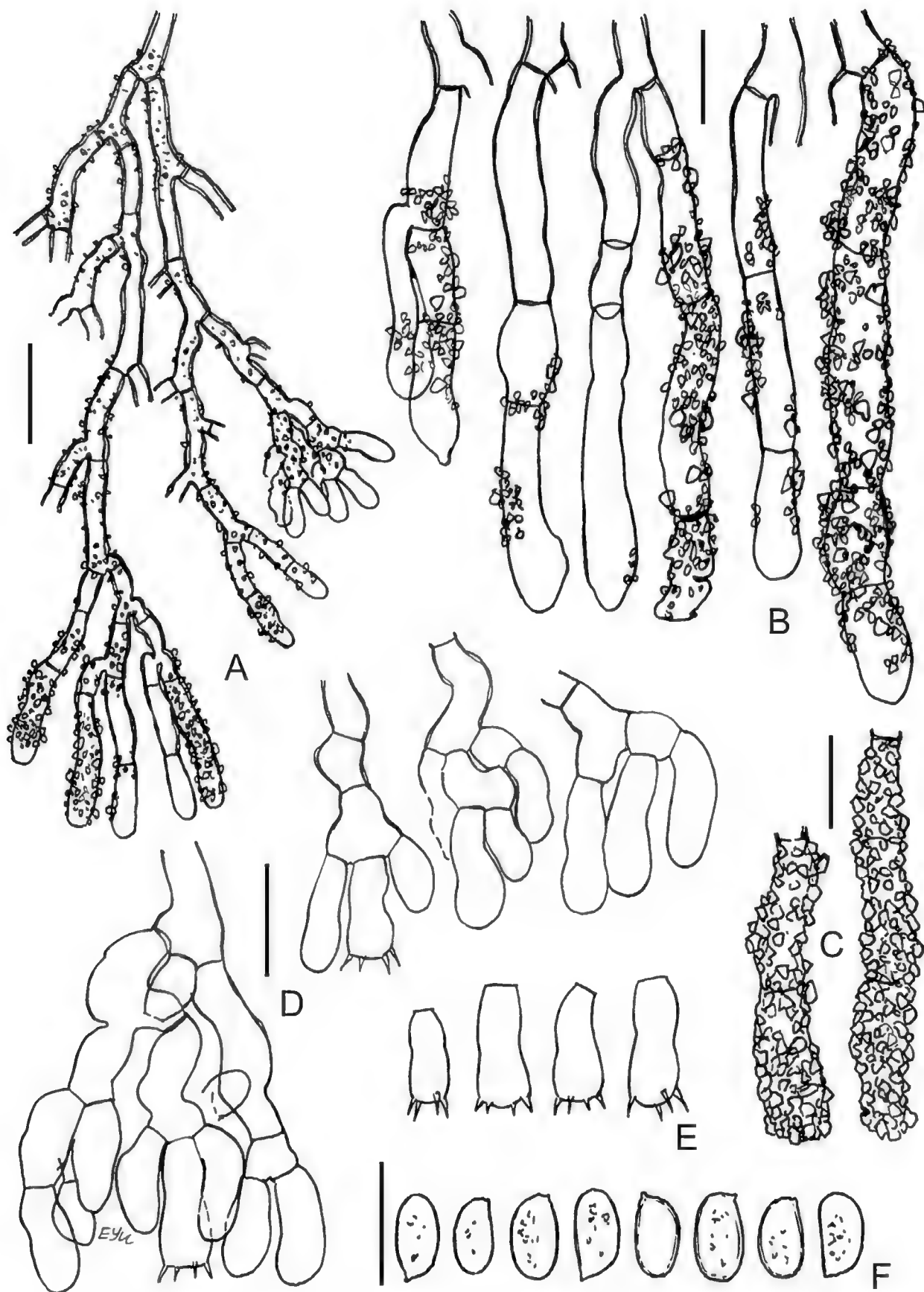
FIG. 6. *Steccherinum filiferum* (holotype, GB-0087369 [KHL 9495]): A. a portion of hyphae from aculeal trama ending by cystidia and basidioles; B. cystidia in KOH; C. cystidia in Melzer's reagent; D. portions of hymenium and subhymenial hyphae; E. basidia; F. basidiospores. Scale bars: A = 20 μm; B–E = 10 μm; F = 5 μm.

Hʏᴘʜᴀʟ sʏsᴛᴇᴍ monomitic, hyphae simple-septate, colorless to pale yellow, smooth or loosely encrusted. Hʏᴘʜᴀʟ ᴄᴏʀᴅs present between fruitbody patches and in subiculum, yellowish or ochraceous, 15–100(–150) μm diam; consisting of 2–40 hyphae, individual hyphae 3.3–8.5 μm wide. Sᴜʙɪᴄᴜʟᴀʀ ʜʏᴘʜᴀᴇ (3–)3.5–6 μm diam, with some segments inflated to 8.5 μm, often branched at right angles or nearly so, thin- to thick-walled, walls up to 1.5 μm thick and appearing layered. Sᴜʙʜʏᴍᴇɴɪᴀʟ ʜʏᴘʜᴀᴇ 2.5–5 μm diam, thin-walled, short-celled.

Cʏsᴛɪᴅɪᴀ (projecting hyphae) in aculei with 0–2 septa, in upper part cylindrical or slightly clavate, rarely submucronate, 4–6 μm wide, richly encrusted by isodiametric bipyramidal crystals 1–3 μm in size in Melzer's reagent, loosely encrusted in KOH, rarely naked, colorless or pale yellowish.

Bᴀsɪᴅɪᴏʟᴇs ovoid, short clavate or short cylindrical, 6–13.5 × (3.2–)4–5 μm, colorless. Bᴀsɪᴅɪᴀ short clavate or subcylindrical, 10–13 × 4.5–4.7 μm, thin-walled, colorless, smooth or barely encrusted; sterigmata four, minute, about 1.5 × 0.3 μm. Bᴀsɪᴅɪᴏsᴘᴏʀᴇs narrowly elliptic, oblong or short cylindrical, 3–3.5(–4) × 1.7–2.3 μm (L = 3.2 μm, W = 1.9 μm in holotype), Q = 1.7–2, thin-walled (appearing slightly thick-walled at ×1000 magnification), sometimes guttulate, inamyloid, acyanophilous; apiculus small or indistinct.

Eᴄᴏʟᴏɢʏ & ᴅɪsᴛʀɪʙᴜᴛɪᴏɴ—So far known from Puerto Rico and Ecuador; inhabits dead branches and remains of palm leaves in evergreen moist subtropical forest and tropical rainforest.

Aᴅᴅɪᴛɪᴏɴᴀʟ sᴘᴇᴄɪᴍᴇɴs ᴇxᴀᴍɪɴᴇᴅ—**ECUADOR, Oʀᴇʟʟᴀɴᴀ Pʀᴏᴠɪɴᴄᴇ, between Puerto Francisco de Orellana and El Dorado**, right bank of the Napo River, 0.4851S, 76.9495W, 260 m a.s.l., rainforest, on fallen woody stem, leg. E. Yurchenko, 20.VII.2019, EYu190720-59 (BLS M-5227); **vicinity of Flor de Oriente**, 0.5042S 76.9819W, 295 m a.s.l., rainforest, on dead rachis of a palm leaf, leg. E. Yurchenko, 21.VII.2019, EYu190721-39 (BLS M-5230; GenBank OP279612); on fallen twig, leg. E. Yurchenko, 21.VII.2019, EYu190721-42 (BLS M-5228); on decaying fallen palm leaf, leg. E. Yurchenko, 21.VII.2019, EYu190721-43a (BLS M-5229).

Cᴏᴍᴍᴇɴᴛs—Most basidiomata in specimens from Ecuador are small, 0.5–3 mm in extent, exceptionally up to 15 mm. The substratum under and around the basidiomata is covered by hyphal cords, especially numerous and notable in BLS-M 5228 (Fɪɢ. 4B). Three of four Ecuadorian specimens (BLS M-5228, 4229, 5230) were found within one 400 m² sample plot.

Steccherinum laxum (L.W. Mill.) K.H. Larss. & Yurchenko, **comb. nov.**
MB 845419

≡ *Odontia laxa* L.W. Mill., Mycologia 26(1): 18 (1934).

≡ *Odonticium laxum* (L.W. Mill.) Ryvarden, Norweg. J. Bot. 25: 296 (1978).

Specimens examined–*Steccherinum laxum*: **USA, ?Ohio**, 'on elm', leg. Morgan, 30.VII.1893 (GB 19282, **isotype** of *Odontia laxa*); **Tennessee, Cosby**, Great Smoky Mountains National Park, along Snake Den Ridge Trail, mixed hardwood-*Thuja* forest, on decaying unidentified wood, leg. K.H. Larsson, 14.VII.2004, KHL 12268 (GB, as *Odonticium laxum*; GenBank JN710577, JN710642, JN710694, JN710729).

A key to *Steccherinum* sensu lato species with simple-septate hyphae

1. Hyphal system monomitic, pseudocystidia absent 2
1. Hyphal system dimitic, pseudocystidia present 5
2. Basidioma stipitate; cystidial elements absent; hyphae more or less constricted at septa .... ***S. minutissimum***
Maas Geesteranus (1974) noted that this species is not a true *Steccherinum* but did not give an opinion about its generic position.
2. Basidioma effused; cystidial elements present or absent; hyphae rarely slightly constricted at septa 3
3. Hymenophore poroid; cystidial elements absent .... ***S. fragile***
3. Hymenophore minutely odontioid; cystidial elemens present 4
4. Aculei in the upper part bearing cylindrical, one-celled or septate cystidia, more or less encrusted by small, loosely attached crystals, partly dissolving or detaching in KOH .... ***S. filiferum***
4. Aculei in upper part bearing numerous, smooth hyphoid elements, and scarce, one-celled or septate cystidia, more or less encrusted by large, plate-like, broadly attached crystals, stable in KOH .... ***S. laxum***
5. Basidioma effused-reflexed, spores 3–5.3 × 2.5–3.5 μm .... ***S. rubigimaculatum***
5. Basidioma effused; spores 2.5–4 × 1.3–2.5 μm 6
6. Basidioma easily detachable from the substratum; aculei up to 0.5 mm long, with conspicuous short-celled terminal hyphae .... ***S. subcrinale***
6. Basidioma closely adnate; aculei 0.1–0.3 mm long, without short-celled terminal hyphae 7
7. Basidioma coriaceous, tough; spores oblong-ellipsoid or short cylindrical, 1.3–1.5 μm wide .... ***S. aridum***
7. Basidioma soft; spores ellipsoid or broadly ellipsoid, 2–2.2 μm wide
.... ***S. cremeoalbum***

Discussion

The first collection of *Steccherinum filiferum* (the holotype collection, KHL 9495) was tentatively identified as a *Candelabrochaete* s.lat., due to its monomitic simple-septate hyphal system and the septate, cystidium-like hyphae at the apex of the aculei. *Candelabrochaete* is a paraphyletic genus (Justo & al. 2017, Li & al. 2022) typified by *C. africana* Boidin, described from Central African Republic. Available sequence of *C. africana* (from Puerto Rico and may not be conspecific with African collections) places it in an isolated position within the *Polyporales*, far removed from the *Steccherinum* clade (Miettinen & al. 2012, Justo & al. 2017). The cystidia in *S. filiferum* may be interpreted as hyphal ends originating from the aculeal trama and not morphologically homologous to septocystidia in *C. africana*. We considered placing the new species in *Odonticium* also, because of its monomitic hyphal system and simple septa. However, this genus is typified by *O. romellii* (S. Lundell) Parmasto, a distinct species with a dense trama and allantoid spores, that belongs in the *Hymenochaetales* (Larsson & al. 2006, Liu & al. 2019).

In their study of the *Steccherinaceae*, Miettinen & al. (2012) showed that *Steccherinum* s.lat. is polyphyletic and discussed the difficulty of defining a monophyletic *Steccherinum* s.str. Both *S. filiferum* and *S. laxum* have morphological features that are atypical for *Steccherinum*, a monomitic hyphal system and loose, open texture of subiculum. They form a strongly supported lineage within the *Steccherinum*-clade (Figure 4 in Miettinen & al. 2012). Although it may be defensible to create a new genus to accommodate these morphologically deviant taxa, following this line of reasoning would necessitate the creation of genera for *Steccherinum tenue* Burds. & Nakasone, *Steccherinum collabens* (Fr.) Vesterh., *Steccherinum nitidum* complex, and other strongly supported clades with typical *Steccherinum* characters. We believe, however, that the best solution is to include the *S. laxum* clade in *Steccherinum* s.lat. rather than divide the genus into many, smaller and morphologically unrecognizable genera.

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Boletus vigasensis sp. nov. an edible species from coniferous forests of Veracruz, Mexico

L. Montoya[1], A. Ramos[1], E. César[2], D. Ramos[1], V.M. Bandala[1*]

[1] *Red Biodiversidad y Sistemática, Instituto de Ecología A.C.*
    Carretera antigua a Coatepec 351, El Haya, 91073 Xalapa, Veracruz, México.
[2] *Herbario XAL, Instituto de Ecología A.C.*
    Carretera antigua a Coatepec 351, El Haya, 91073 Xalapa, Veracruz, México.

[*] Correspondence to: *victor.bandala@inecol.mx*

Abstract—A new *Boletus* species belonging to the Aereus group was found among specimens collected in coniferous forest in the Cofre de Perote Mountain area in central Veracruz State; and also among specimens obtained in a local traditional market in the city of Xalapa, Veracruz, where such fungi are sold and used by the locals and offered in restaurants. Morphological and molecular analyses of the species are presented, accompanied with photographs and line drawings.

Key words—American boletes, ectomycorrhizal mushrooms, wild edible mushrooms, porcini mushrooms

Introduction

There are currently around 2486 names of species identified within *Boletus*, but due to molecular systematics research made in the last decade, this number has been reduced to about 1245, corresponding to around 800 accepted species distributed worldwide (http://www.indexfungorum.org). Over time, various lineages (sections) in the genus have been recognized, and based on the application of molecular studies, *Boletus* sect. *Boletus* (the "porcini" clade s.str.) together with five lineages (Aereus, Alloboletus, Inferiboletus, Obtextiporus, and Variipes; not yet formally described) are currently accepted (Dentinger & al. 2010, Feng & al. 2012, Nuhn & al. 2013, Cui & al. 2016).

It is well known that the edible bolete species around *Boletus edulis* Bull. have been collected and consumed since ancient times (Hall & al. 1998, Leonardi & al. 2005, Sorrenti 2017), being among the most collected edible wild mushrooms and considered a culinary delicacy with significant commercial demand worldwide (Singer 1986, Boa 2004, Arora 2008, Sitta & Davoli 2012). Species related to *B. edulis* are found to a wide extent throughout the Northern Hemisphere (Lakhanpal & al. 1988, Yamanaka & al. 2000, Nounsi & al. 2014, Outcoumit & al. 2014, Bozok & al. 2020), and some of them have been introduced in New Zealand and South Africa (Wang & al. 1995, Hall & al. 1998). Additionally, *B. albobrunnescens* Desjardin & al. has recently been recognized as endemic to Thailand and *B. austroedulis* Halling & N.A. Fechner as endemic to Australia (Halling & al. 2014).

Wild mushroom species morphologically resembling European *B. edulis* have been consumed in Mexico since pre-Hispanic times. These are recognized in Spanish terms as "panzas", "pancitas", "cemitas", etc., and in various names in native languages, for example "sekub t´ul" (Tsotsil), "serochaco", "sonaka" (Raramuri), "jopante", "Jthä", (Otomí), "baya retni", "mey guiet xtil" (Zapotec), "pante", "xotoma", "xotomate", "pananácatl" (Náhuatl), among others (Herrera & Guzmán 1961, Guzmán 1997, Jarvis & al. 2004, Garibay-Orijel & al. 2009, Lara & al. 2013, Quiñónez-Martínez & al. 2014, Ruan-Soto 2018, Jaime 2019, Montoya-Esquivel & al. 2003, 2019). In the region of Xalapa, Veracruz, and possibly in other areas of Mexico, due to foreign influence, the name "porcini" began to be part of the language among merchants and has spread gradually in traditional markets by the local vendors. It is evident that the culinary use of that group of mushrooms in urban areas of the country has a notable increase following the trends of international cuisine.

Mexico is diverse in terrestrial ecosystems, and edible species of *Boletus* are collected in many regions, but there is special emphasis on this activity in the central zone, along the Trans-Mexican Volcanic Belt (Herrera & Guzmán 1961, Mapes & al. 1981, Bandala & al. 1997, Montoya-Esquivel & al. 2003). In this vast mountain range are the highest peaks of the country, including important forest areas such as the Cofre de Perote National Park, which includes an extinct volcano (Cofre de Perote or Nauhcampatépetl [its Náhuatl name]) that reaches 4282 m in altitude. This area is located 64 km away from the city of Xalapa (Veracruz state capital) and is where our collections of *Boletus* specimens were made. This mountain hosts coniferous forests and oak-conifer forests that favor

the development not only of *Boletus* species but also of other edible fungi. At the site, an important folk knowledge about the use of around 53 edible species of mushrooms is culturally conserved; such fungi are harvested and form part of the "basic family basket of edible natural products", and even represent a source of income for the locals (Bandala & al. 1997).

The taxonomy and classification of the species in the *Boletus edulis* group, mainly due to their economic importance in different regions of the world, is gradually being revised (Dentinger & al. 2010, Feng & al. 2012, Halling & al. 2014, Cui & al. 2016, Gelardi 2020) to the point that mycelium of the species is being certified, with morphological and molecular support, for long-term reforestation programs that increase the production of sporocarps (Mello 2012). Despite the widespread use of sporocarps of *B. edulis* group and even of other edible fungi in Mexico, little taxonomic research has been conducted to estimate their specific diversity in the country. Most records have often focused on some macroscopic characters, such as size, form, taste, and color. We have approached the taxonomic revisions of apparently well-defined Mexican macrofungi species, which have been commonly cited under names of European or American (USA) species but which in fact, correspond to other taxa. Obviously, such broad concepts underestimate the diversity of Mexican species. Furthermore, to generate suitable protocols for their commercial sale, we consider the identification of wild edible mushrooms as a basic task, which would help to position specific taxa within the global market, trace their natural and commercial distribution, facilitate the allocation of prices, and guarantee hygiene and food safety. In addition, proper identification will help to protect their populations. In this work, we review the status of collections from the Cofre de Perote Mountain superficially similar to *B. edulis*, a site where their fruiting and harvesting are common. Fruit bodies purchased from local markets, where they are popularly traded, were also included — they come from this mountain, according to the traders. A morphological study together with molecular evidence implies the existence of more than one species, but here we focus just on the one for which we had best support for recognition as a new species. It presents all the characters defining *Boletus* and, in our phylogeny (Fɪɢ. 1), it is distant from the *B. edulis* clade, but related to the Aereus group. This species is proposed here as a new species, *Boletus vigasensis*, which is described, illustrated, and taxonomically discussed.

Materials and methods

Sampling and morphological study

Basidiomes of specimens of *Boletus* superficially resembling those of "porcini" fungi were collected during 2015 and 2018 in the Natural Protected Area "San Juan del Monte", located in the municipality of Las Vigas, on the Cofre de Perote Mountain, in Veracruz State (Eastern Mexico), of the Trans-Mexican Volcanic Belt, at an altitude of 2520–2770 m, in mixed pine forests of *Pinus patula* Schltdl. & Cham., *P. pseudostrobus* Lindl., and *P. teocote* Cham. & Schltdl. The climate varies from cold subhumid to mild subhumid, with annual average temperatures between 12–18°C, and 1000–1800 mm average annual rainfalls. In the city of Xalapa, 64 km east of Cofre de Perote, we also bought some fruitbodies at the Alcalde y García traditional market which were harvested on that mountain.

Descriptions of colors, macromorphological, and organoleptic characteristics were gathered from fresh samples; color records follow Munsell (1994; e.g., 2.5Y8/3) and Kornerup & Wanscher (1967; e.g., 5B5). The preservation, micromorphological study, and the line drawings were developed according to Montoya & al. (2019). In the descriptions, Xm denotes the mean values of length and width of basidiospores per collection in n collections and its standard deviation, and Qm refers to the range of coef. Q (where Q is the average of the ratio length/width of basidiospores in each collection). Collections are deposited in the Herbarium of Instituto de Ecología A.C., Xalapa, Veracruz, México (XAL).

DNA extraction, PCR and sequencing

DNA extraction was performed according to César & al. (2018). Following previous research on the genus *Boletus* (Leonardi & al. 2005, Dentinger & al. 2010, Cui & al. 2016, Wu & al. 2016, Chakraborty & al. 2017, Sarwar & al. 2018, Tremble & al. 2020), the internal transcribed spacer (ITS) region of ribosomal DNA was extracted and amplified according to previous protocols, using the primers ITS1F, ITS5/ITS4 (White & al. 1990, Gardes & Bruns 1993). Sequencing of the amplified PCR products was performed on a Genetic Analyzer 3730XL sequencer (Applied Biosystems). All the sequences obtained were edited, assembled, and deposited in the GenBank database (Benson & al. 2017). Accession numbers are indicated in Fig. 1.

Phylogenetic analysis

With the sequences obtained, we developed a similarity analysis from GenBank, using the BLAST program. The sequences produced in this work (Fig. 1) together with all the ITS sequences available in the GenBank (GB) database named as *Boletus* (1078 sequences), were used to build a data set using the PhyDE v.0.9971 program (Müller & al. 2010). The alignment was carried out with the MAFFT online service program (Katoh & al. 2019), and the inconsistencies were corrected manually. The phylogenetic tree was generated (data not shown here), and the evolutionary model was calculated using the IQTree v2.1.1 package (Nguyen & al. 2015, Kalyaanamoorthy & al. 2017, Minh & al. 2020), and the best-fit model, using the Bayesian Information Criterion

(BIC), the Akaike Information Criterion (AIC), and corrected AIC. The latter was used to generate a phylogenetic tree with the Maximum Likelihood (ML) method, with a Nearest Neighbour Interchange (NNI) heuristic, with a specific evolutionary model for data set. By default, the IQTree program generated a consensus tree by calculating the Robinson-Foulds distance between the ML tree and the consensus tree, with the branches being tested by means of Ultrafast Approach Bootstrap (UFBoot), SH-like approximate Likelihood Ratio Test (SH-aLRT), Approximate Bayes test (aBayes) and Bootstrap Standard (BS). After a second optimized alignment of the entire genus *Boletus*, an inferred phylogenetic tree with high molecular support was generated being consistent and the same clades are presented in the tree of Fɪɢ. 1. Another phylogenetic tree was generated also by Bayesian Inference (BI), using MrBayes v. 3.2.7 (Ronquist & al. 2012). The phylogenies from ML and BI analyses were displayed using FigTree v1.4.4 (Rambaut 2018). Being consistent with both phylogenetic trees (ML phylogenetic tree and BI phylogenetic tree), the Bayesian posterior probability values are presented in the branches of Fɪɢ. 1.

Results

In the phylogeny obtained, the sequences of the two new Mexican specimens formed an independent and strongly supported clade. Macromorphological features such as pileus and tube color, and white to staining pale brown to lilaceous context, combined with micromorphological features such as size of basidiospores and cystidia, and a well differentiated ixotrichoderm are distinctive taxonomically from close relatives to consider that the samples represent a new species. In the phylogeny obtained it appears related to *B. occidentalis* B. Ortiz & T.J. Baroni described from Dominican Republic, *B. atkinsonii* Peck from USA, and *B. barrowsii* Thiers & A.H. Smith from USA and Mexico.
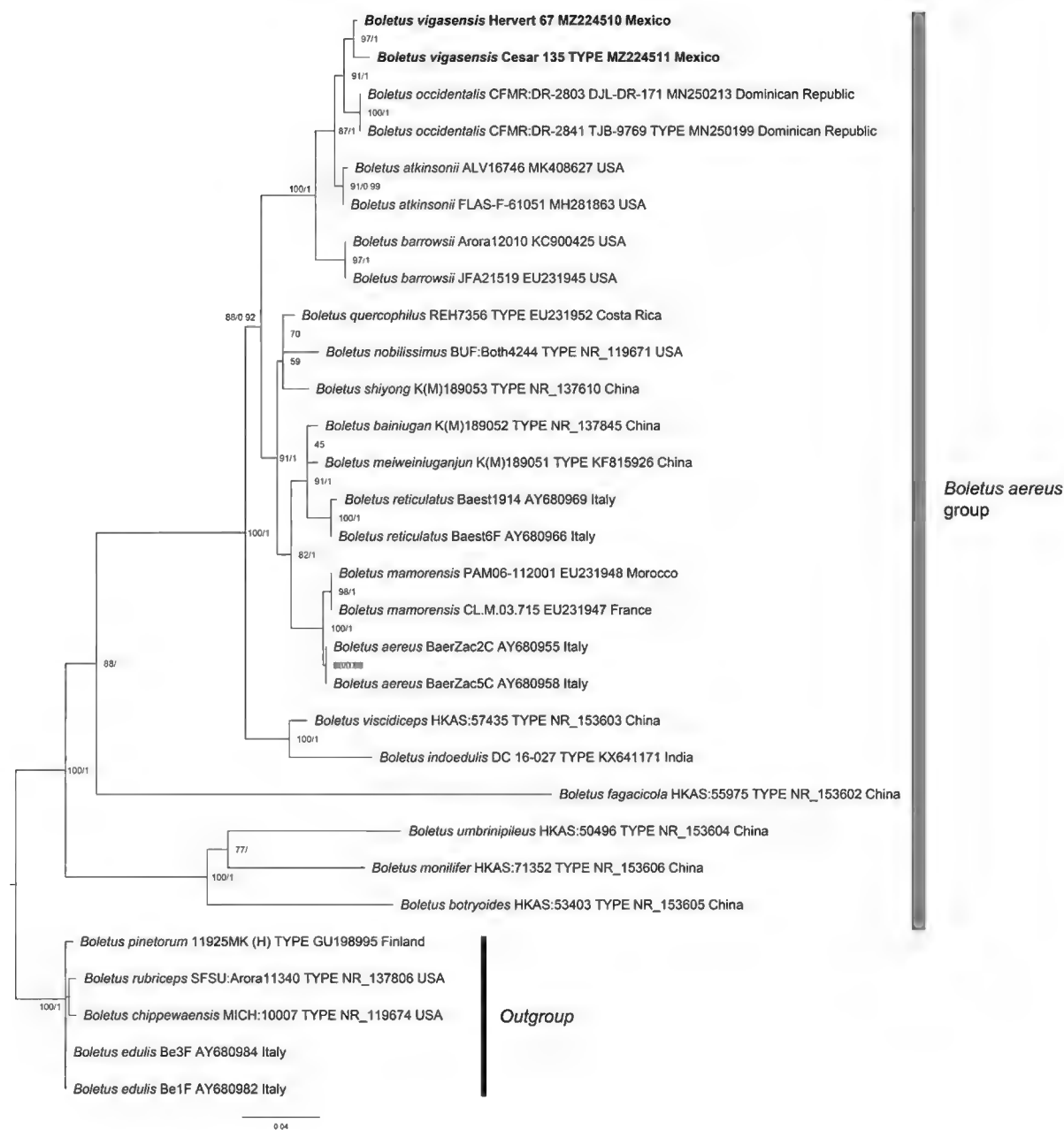
FIGURE 1. Phylogenetic relationships within *Boletus* species inferred from the ITS sequences by maximum likelihood method. The new species is indicated in bold letters. Bootstrap scores ≥70% and Bayesian Posterior Probabilities ≥0.90 are indicated above the branches.

Taxonomy

Boletus vigasensis Montoya, E. César, Ant. Ramos & Bandala **sp. nov.**   FIGS 2–4

MB 845787

Differs from other *Boletus* spp. by its pale brownish to greyish-orange pileus, its context white staining brown to violaceous, its mustard-brown to orange-brown pores, its basidiospores 13–16.5(–19.5) × 4.5–5 μm, its long pleurocystidia, and its pileipellis as a slightly gelatinized trichoderm.

Type. —Mexico, Veracruz, Cofre de Perote mountain, Municipality of Las Vigas, San Juan del Monte, 19.6147°N 97.1117°W, 2528 m.a.s.l., solitary near *Pinus pseudostrobus* in a mixed pine forest with *Pinus patula* and *P. teocote*, 29 August 2018, César 135 (Holotype, XAL; GenBank ITS MZ224511; 28S MZ198357; rpb1 MZ233731).

Etymology—The epithet refers to Las Vigas, the town closest to the San Juan del Monte protected natural area.

Pileus 41–116 mm diam., subhemispheric, convex to plano-convex when mature, surface regular, sometimes slightly cracked, somewhat punctate pruinose, soft, at times with detachable cuticle, surface pale brown (2.5Y 7/4), pale greyish-orange (10YR 5/4-4/4; 6D5), pale brown (10YR 4/6; 5D7, 5D5), below pileus cuticle pale pinkish-brown (7.5YR7/2; 6B2); when young with incurved margin, straight or undulate when mature. Hymenophore plane to slightly convex. Tubes 18–35 mm in length, straight, adnate, greyish (2.5Y 7/2, 7/3; 4B2) when young, changing to pale mustard-yellow (2.5Y7/6; 4B5), unchanging when bruised. Pores <1 mm diam, round to slightly angular, pale mustard-brown (2.5Y6/8; 4B7) to cinnamon-brown or faintly orange (10YR5/6, 6C6), when bruised dark brown (10YR3/1; 6F4), with a whitish layer of tangled hyphae covering the pores (stuffed pores) at primordia or in immature specimens. Stipe 70–121 × 31–49 mm, clavate to subclaviform, straight or curved, attenuated towards the pileus attachment, broadened towards the base; compact, dull, fibrillose, with a fine and moderately open mesh from the top to the middle of the stipe, which appears pale greyish-beige (2.5Y6/4) to chocolate-brown (10YR 4/4), in the primordia the apex is dark greyish-beige (2.5Y5/4; 5C4) to pale beige (2.5Y 6/4–7/4), with a white base. Context 9–20 mm broad, whitish, compact, staining pale brown (10YR 7/3; 5B3) to vinaceous-lilaceous (7.5YR6/3; 7C3) especially towards the cuticle. Odor fungoid, mild, somewhat similar to steam cooked vegetables. Taste mild, fungoid, agreeable.

Macrochemical test: pileus surface slightly reddish to violaceous with NH$_4$OH vapors.
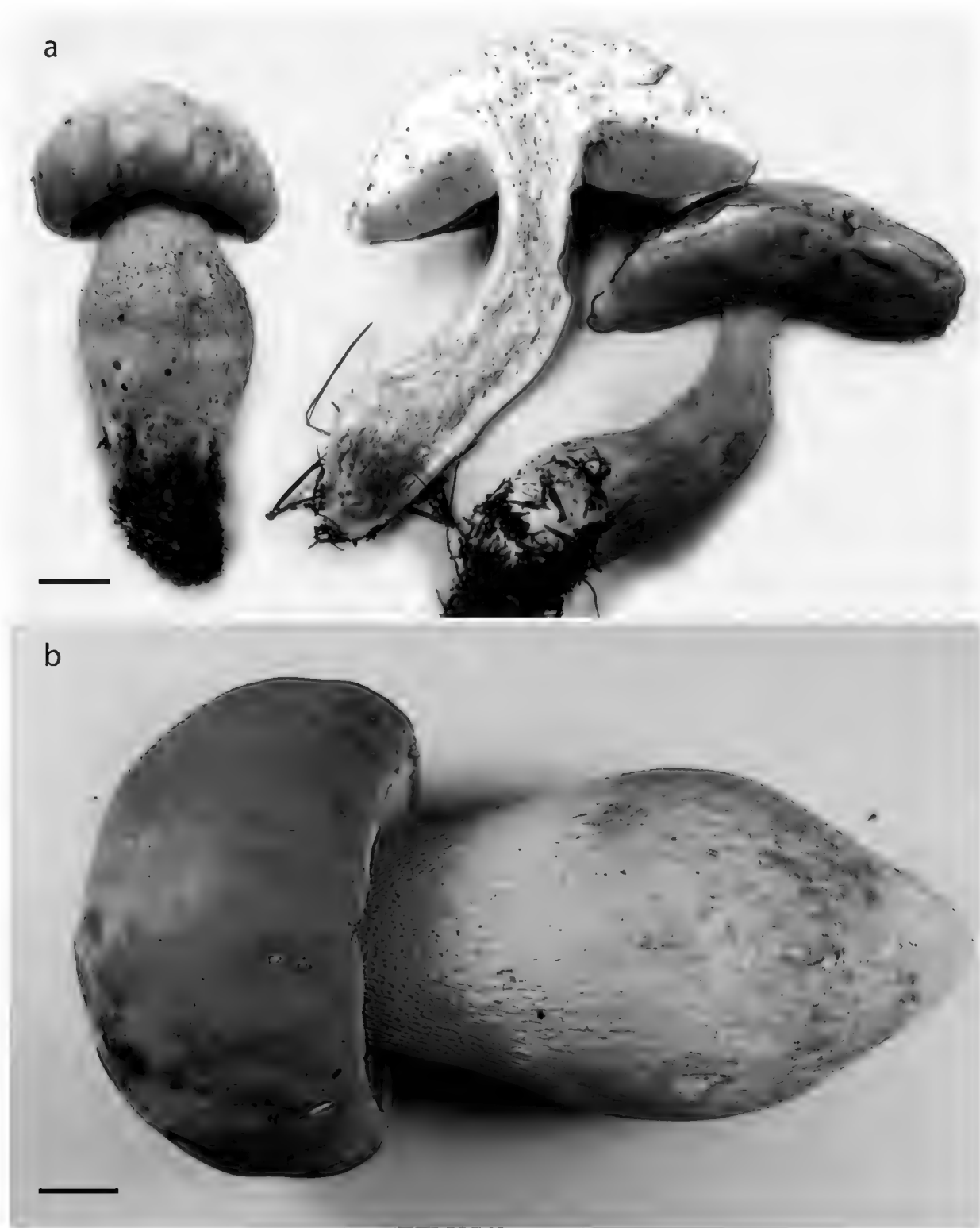
FIGURE 2. *Boletus vigasensis*: basidiomes: a. XAL–César 135 (holotype); b. XAL–Hervert 67. Scale bars: a = 20 mm; b = 10 mm.

BASIOSPORES 13–16.5(–19.5) × 4.5–5 μm, Xm = 14.86 ± 1.50 × 4.56 ± 0.17, Qm = 3.25 ± 0.31, fusiform to narrowly ellipsoid, with slight suprahilar depression, pale yellowish, smooth; wall up to 0.5 μm thick. Basidia 25–37(–41) × 9–13 μm, commonly tetrasporic, at times tri- and bisporic, thin walled, hyaline. PLEUROCYSTIDIA 46–62 × 7–13 μm, narrowly lageniform, hyaline, thin

walled, infrequent. CHEILOCYSTIDIA 24–47 × 5–10 μm, like pleurocystidia, some narrowly subutriform or apically tortuous other clavate, hyaline, at times pale yellowish, thin walled, infrequent. CAULOCYSTIDIA 27–62 × 6–10 μm, narrowly lageniform or broadly lageniform some subcylindrical, often somewhat tortuous, hyaline, thin walled, at times thick-walled (c. 1 μm thick). PILEIPELLIS a slightly gelatinized trichoderm, formed by elements disposed in chains, mostly grouped in cumulus, intercalary elements 4–10 μm diam, pale yellow, hyaline, thin-walled, some collapsing; terminal elements 17–62 × 6–10(–13) μm, lageniform, subfusiform, lecythiform or at times subcylindrical, thin walled, hyaline. Clamp connections absent.

ECOLOGY & DISTRIBUTION— In coniferous forest, on soil, growing under *Pinus pseudostrobus* in a mixed pine forest with *P. patula* and *P. teocote.*

> ADDITIONAL SPECIMEN EXAMINED—**MEXICO, VERACRUZ, Xalapa**, Alcalde y García market, 12 November 2015, Hervert 67 (XAL).

COMMENTS—In the phylogeny (FIG. 1) *Boletus vigasensis* appears showing relationship among the species of the Aereus group, sister to *B. occidentalis*, which differs by its dark brown pileus when young becoming yellow brown, the unstaining white context, pores soon yellow green to olivaceous with age, the stipe with a coarse white to pale tan reticulum at the top, shorter basidiospores (11.2–15.2 × 4–4.8 μm), shorter pleurocystidia (37.6–48 × 4.8–8 μm), and pileipellis having a subgelatinous tangled layer of repent to suberect hyphae (Ortiz-Santana & al. 2007). *Boletus atkinsonii* and *B. barrowsii* are also closely related to *B. vigasensis* (FIG. 1). However, according to the protologue (Peck 1905) *B. atkinsonii* has paler (yellow or subochraceous) pores, brownish stipe reticulum and shorter basidiospores (10–12.7 × 4–5 μm, Peck 1905; 10–14 × 3.5–4.5 μm, Smith & Thiers 1971). Basidiomes of *B. barrowsii*, according to the protologue (Thiers 1976) reach a bigger size, with the pileus up to 300 mm and with "pale pinkish buff" to "pinkish buff" colors during all stages of development, the tubes shorter (10–20 mm long) with wider pores (1–2 mm width), the stipe bulbous (bulb up to 90 mm broad over the base) with a typically fine and delicate reticulum often all the way to the base, unstaining context, basidiospores shorter (13–15 × 4–5 μm), cystidia shorter (37–50 × 5–9 μm), and the pileus cuticle disposed in a poorly differentiated cutis. In *B. vigasensis* the pileipellis is a well differentiated ixotrichoderm, corresponding to the type 3 proposed by Cui & al. (2016), and with a context that turns to pale brown or vinaceous-lilaceous when exposed. Other species belonging to group Aereus are *B. bainiugan* Dentinger from China, with a pileipellis in a trichoderm but without a gelatinous matrix and with frequently branched

elements; and *B. quercophilus* Halling & G.M. Muell. from Costa Rica, with yellow colored pileus, subtomentose to scaly-fibrillose at margin, and with shorter basidiospores (Dentinger & Suz 2014, Halling & Mueller 1999).
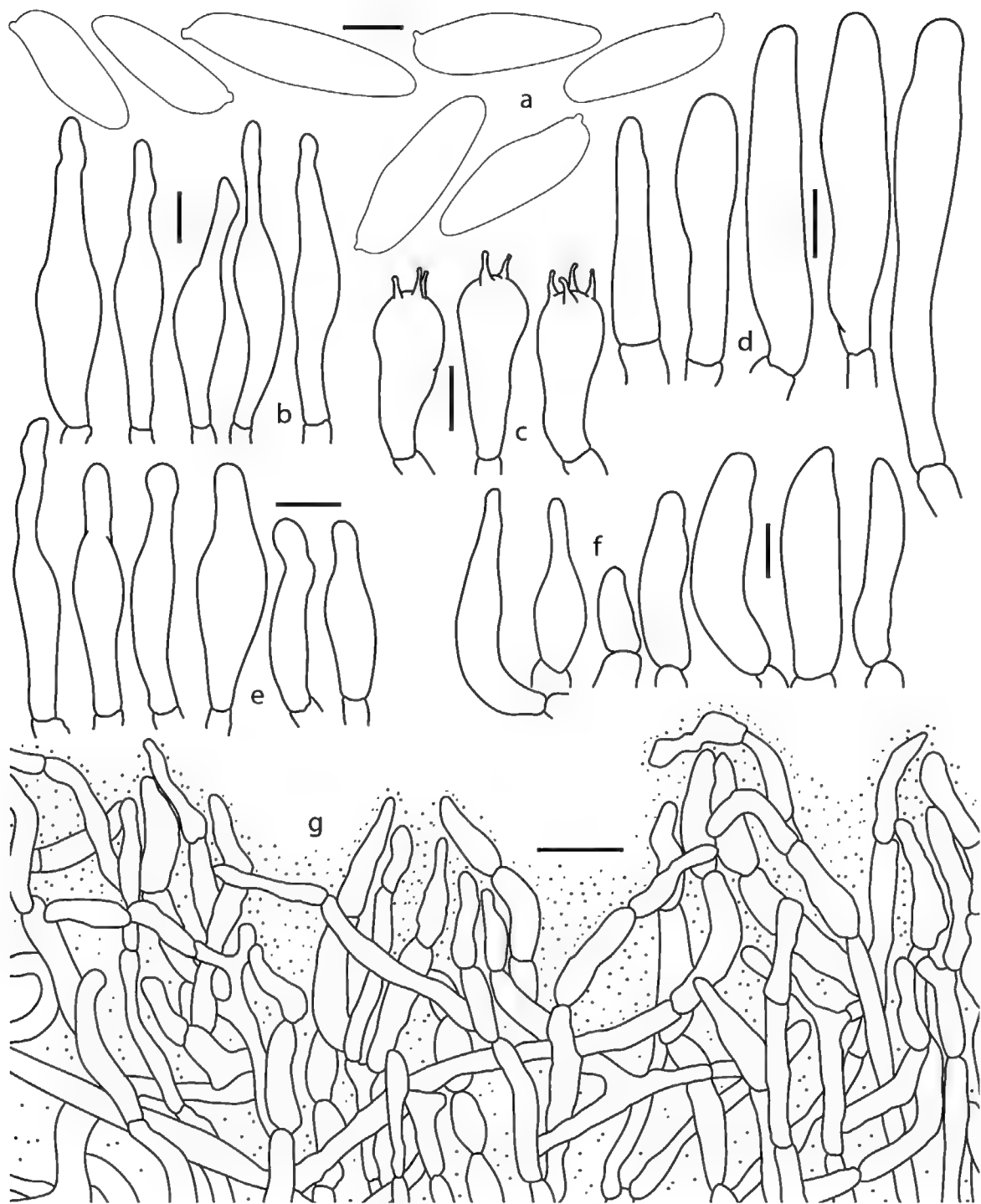

Figure 3. *Boletus vigasensis* (holotype, XAL–César 135): a. basidiospores; b. pleurocystidia; c. basidia; d. caulocystidia; e. cheilocystidia; f. pileipellis terminal elements; g. pileipellis. Scale bars: a = 5 μm; b–f = 10 μm.
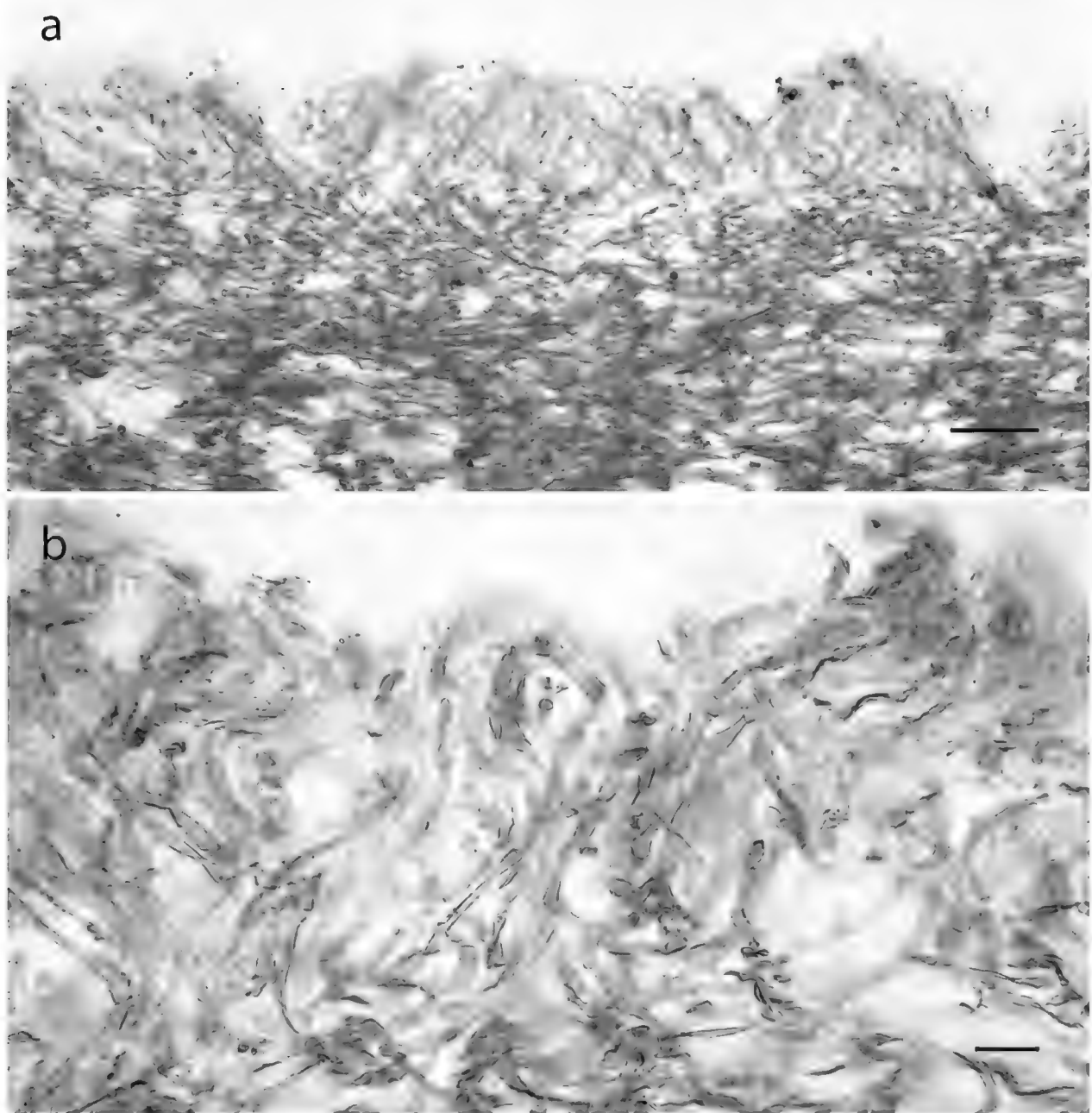
Figure 4. *Boletus vigasensis* (holotype, XAL–César 135): pileipellis. Scale bars: a = 100 µm; b = 25 µm.

Key to *Boletus vigasensis* and species of the Aereus group and *B.* sect. *Boletus*

(The Aereus group spp. are phylogenetically close to *B. vigasensis*; and the *B.* sect. *Boletus* spp. are morphologically similar to *B. vigasensis*)

1. Pileus developing ferruginous to vinaceous-tawny tinges 2
1. Pileus color not as above 4
2. Pores staining reddish-brown when bruised, with conspicuous hymenial cystidia 3
2. Pores and context above tubes unstaining when bruised but may show slight bluing if old or wet; hymenial cystidia inconspicuous ....*B. rubriceps*

3. Pileus pale lemon yellow and decorated irregularly with ferruginous or vinaceous tawny spots; Pores yellowish to pale orange-brown, staining pinkish or vinaceous cinnamon on bruising; Basidiospores up to 16 μm in length, pileus context stains dingy tan...*B. chippewaensis*
3. Pileus with brown coloration, lacking lemon yellow hues and not irregularly marked with vinaceous spots; pores white when young, coloring with age greenish yellow, not or slightly darkening on bruising; basidiospores up to 19 μm in length...*B. edulis*
4. Pileus white to whitish or buff during all stages of development ....*B. barrowsii*
4. Pileus pale brown, grayish brown to yellowish brown, even dark brown.................. 5
5. Pileus dark brown in button stages, becoming paler with expansion ..*B. occidentalis*
5. Not as above 6
6. Context white, pileipellis hyphae roughened; basidiospores up to 14 μm in length .... *B. atkinsonii*
6. Context staining, at least under pileus cuticle 7
7. Context yellowish under the cuticle; pores concolorous with tubes, in yellow to greenish or greyish-yellow colours; pileipellis hyphae brown, some very dark .... *B. pinetorum*
7. Context staining pale brown to vinaceous-lilaceous especially towards the cuticle; pores pale mustard to cinnamon-brown or faintly orange, when bruised dark brown; pileipellis hyphae not as above.. *B. vigasensis*

Discussion

Among the fungi commercialized as the porcini in Xalapa, Veracruz, solely because of the appearance of a robust and relatively large fungus, *B. vigasensis* appears related to *B. edulis*, with which in fact it has some resemblance. *Boletus edulis* is a European fungus, which in the diagnosis (Bulliard 1782) was described as edible, with pileus 250–280 mm diam (10–11 inches diam), with a firm, white, unchanging context, with white tubes when young, coloring with age, with an agreeable odor and taste. Later, the sanctioning work (Fries 1821) defined it with a pulvinate, glabrous pileus; with semi free, roundish, white but soon yellowish tubes, stipe thick and reticulate. These features, however, are not unique to *B. edulis*, but to a greater or lesser degree shared with different similar species of the "edulis complex". It is likely that derived from the popular culinary value and perhaps also from the currently significant commercial value of this mushroom, the name "*B. edulis*" has been used in different regions of the world for wild populations of fungi that at first glance are superficially similar but are taxonomically different; such is the case of *B. vigasensis*, which belongs to the Aereus group, distant from the "species complex" of *B. edulis* (*B.* sect. *Boletus*). This situation has been common to other popular highly

appreciated wild edible mushrooms (Alessio 1985, Mueller 1991, Wang & al. 1995, Guzmán & Ramírez 2001, Pilz & al. 2003, Foltz & al. 2013, Arora & Frank 2014, Huang & al. 2021).

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Mycena cinereoalba sp. nov. from Qinghai province, China

Ting-Ting Qiang[2], Lu-Chao Bai[1,2,*]

[1] *State Key Laboratory of Plateau Ecology and Agriculture*
   College of Agriculture and Animal Husbandry, Qinghai University
   Xining, Qinghai 810016, China
[2] *College of Agriculture and Animal Husbandry, Qinghai University*
   Xining, Qinghai 810016, China.

Correspondence to: Luchao_Bai@outlook.com

Abstract– From specimens collected in Sanjiangyuan (Source of Three Rivers) region, Qinghai Province, China, *Mycena cinereoalba* is described based on its morphological characteristics and a molecular phylogenetic analysis using the internal transcribed spacer (ITS) region and nuclear ribosomal RNA large subunit (nrLSU) sequences. The phylogenetic relationship inferred from the ITS and nrLSU sequence data matrices confirmed that this new species is different from other species of *Mycena* for which DNA sequences are available. The species are comprehensively described and illustrated in detail with photographs. Phylogenetic relationships among the species were analyzed, and two phylograms were provided in this study.

Key words–*Mycenaceae, Agaricales, Basidiomycota*, taxonomy

Introduction

Mycena (Pers.) Roussel is the largest genus in *Mycenaceae* (*Agaricales, Basidiomycota*). It is a widely distributed, resource-rich genus with more than five hundred known species in the world, most of which are saprophytic (Kirk & al. 2008). The main characteristics of this genus are: basidiomata are small; pileus is conical, parabolic or bell-shaped, thin and membranous, with or without stripes on the surface, and nearly smooth, and some species are hygrophanous; cap is thin; lamellae are adnate, decurrent or arcuate; stipe is

cylindrical, hollow, and fragile; spores are smooth and hyaline; the spore print is white; and cystidia are present and intricate. *Mycena* species are usually distributed in coniferous and other woody forests and are rarely found near grasses, mosses, and ferns (Pegler 1986; Singer 1986). Fungi have an important ecological, economic, and scientific research value (Boa 2004; Peters & Spiteller 2007); and in recent years, *Mycena* species have provided a new theoretical basis for the breeding and protection of orchids (Guo & al. 1997, 2019).

During an investigation of macrofungi in the main forested areas of the "Source of Three Rivers" region in Qinghai Province, China, an unidentified species was found at different locations in 2019–20. After further microscopic observation and phylogenetic analysis based on ITS and nrLSU sequences, it is described here as a new species.

Materials and methods

Specimens were collected and color photographs of the habitats of the fresh fruiting bodies were taken after rainy days. To ensure their integrity, ecological, habitat and morphological characteristics were recorded in the field. Some tissues were dried using silica gel in a sealed plastic bag, while others were dried in an electric oven and stored in a –20°C refrigerator at the Herbarium of Plant Pathology, Qinghai University, Xining, China (QHU).

Macromorphological descriptions of the basidiomata, including the size, shape, and surface characteristics of their pileus, lamellae, and stipe, were based on the fieldnotes and photographs. Color codes followed Kornerup & Wanscher (1978).

Micromorphological data were based on observations of the unarmed sections of the dried specimens, including the spores, basidia, pileipellis, and stipitipellis. Small pieces of dried specimens were revived in 5% KOH, stained with 1% Congo Red when necessary, and observed with an Olympus CX31 microscope. Twenty basidiospores and 10 basidia were measured in each collection. Size was indicated as "a–b × c–d". *Q* referred to the ratio of length to width in an individual spore, and *Qm* referred to the mean of the *Q* values.

Genomic DNA was extracted from the dried basidiocarps using the modified CTAB method (Doyle 1987). The primer pairs ITS4 and ITS5 and LR0R and LR7 (White & al. 1990; Vilgalys & Hester 1990) were used for PCR amplification of the ITS and nrLSU, respectively. PCR amplifications were performed with a total volume of 25 μL. The PCR procedure for the ITS was: predenaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing

at 55°C for 30 s, elongation at 72°C for 60 s; and a final extension at 72°C for 10 min. The PCR procedure for the nrLSU was: predenaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 40 s, elongation at 72°C for 1.5 min; and a final extension at 72°C for 10 min. Amplified PCR products were examined by gel electrophoresis using 1% agarose gel. Electrophoresis was conducted in a 1×TAE buffer for 30–40 min with a voltage of 120 V. Purification and sequencing were conducted by Lanzhou Tianqi Gene Biotechnology Co., Ltd. The primers used in the PCR were the same as those used in sequencing. The newly generated sequences have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Related GenBank sequences were identified by BLAST searches and retrieved for analysis. Alignments were constructed separately for gene fragments by the Multiple Alignment using Fast Fourier Transform (MAFFT) software version 7 (https://mafft.cbrc.jp/alignment/server) (Katoh & Standley 2013) and then manually adjusted in BioEdit (Hall 1999). The best base substitution model was obtained by ModelTest (Nei & Kumar 2000; Tamura & al. 2013). A phylogenetic tree was constructed in MEGA 5.2 using the Maximum Likelihood (ML) method with 1000 bootstrap replicates.

Taxonomy

Mycena cinereoalba L.C. Bai & Qiang, **sp. nov.**   Figs 1, 2
MB 841193

Differs from *Mycena pura* by its grayish-white mature pileus, its gray lamellae, and its gray stipe with short fibrous white villi.

Type: China, Qinghai Province, Sanjiangyuan region, Jiangxi Forest Farm, 32.0754°N 97.0020°E, alt. 3501 m, 22 Aug 2020, L.C. Bai & T.T. Qiang (**Holotype**, QHU20461; GenBank OK091026, OK091029).

Etymology: *cinereoalba* refers to the grayish white color of the basidiomata.

Basidiomata small. Pileus 28 mm diam, bell-shaped to hemispherical, nearly applanate when mature, grayish white, with stripes at the edge, hygrophanous, nearly smooth, and sticky when wet. The context is thin and white. Lamellae 3–5 mm broad, unequal, nearly closed, adnate, and nearly arcuate, gray and wrinkled on the surface, with a transverse vein between them, wavy at the edge. Stipe 35 × 6 mm, central, cylindrical, gray, with stripes and short, fibrous white villi on the surface; hollow, fragile and easily cracked, with a white basal mycelium. Odour, like carrots, though not distinctive.
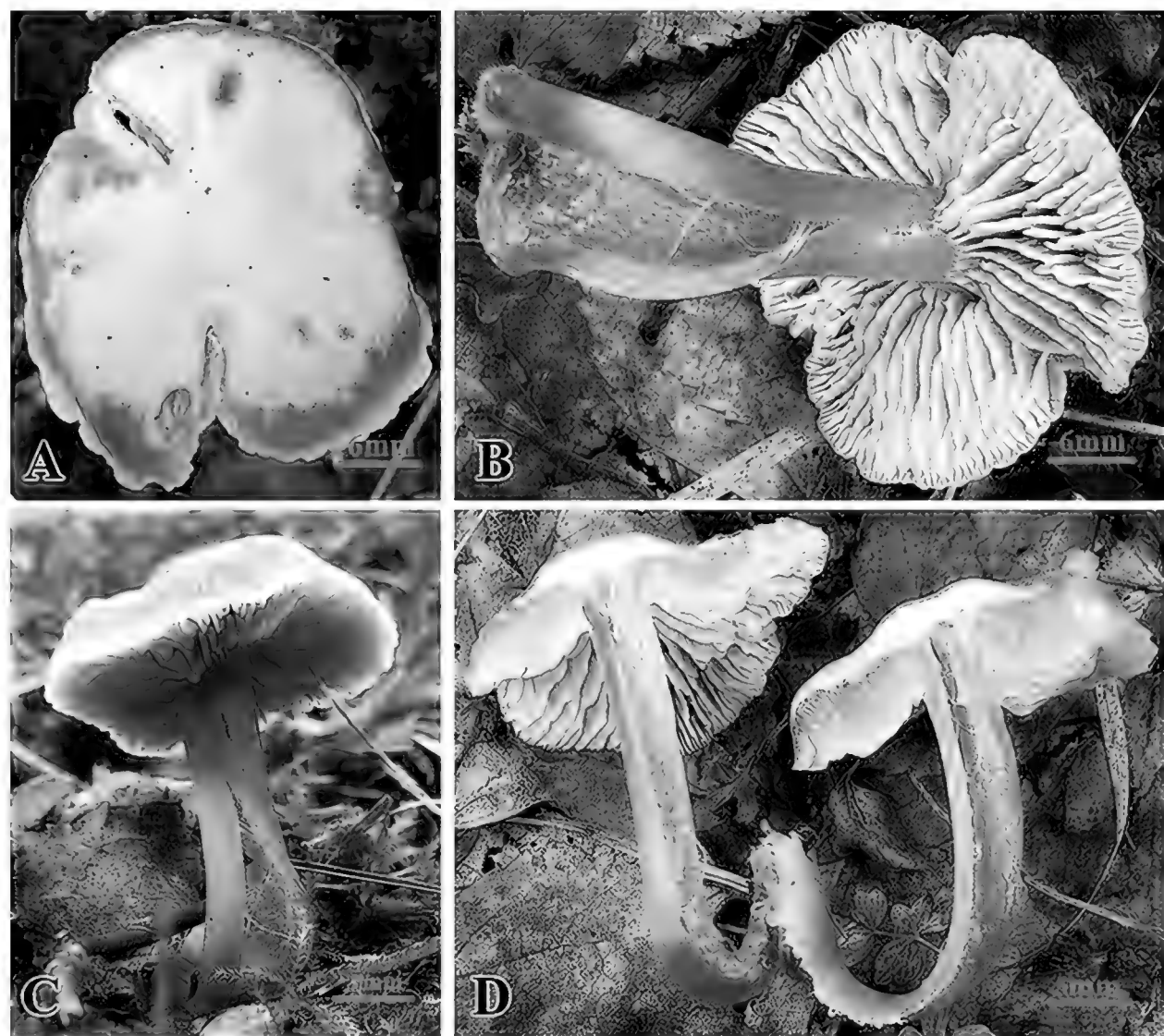
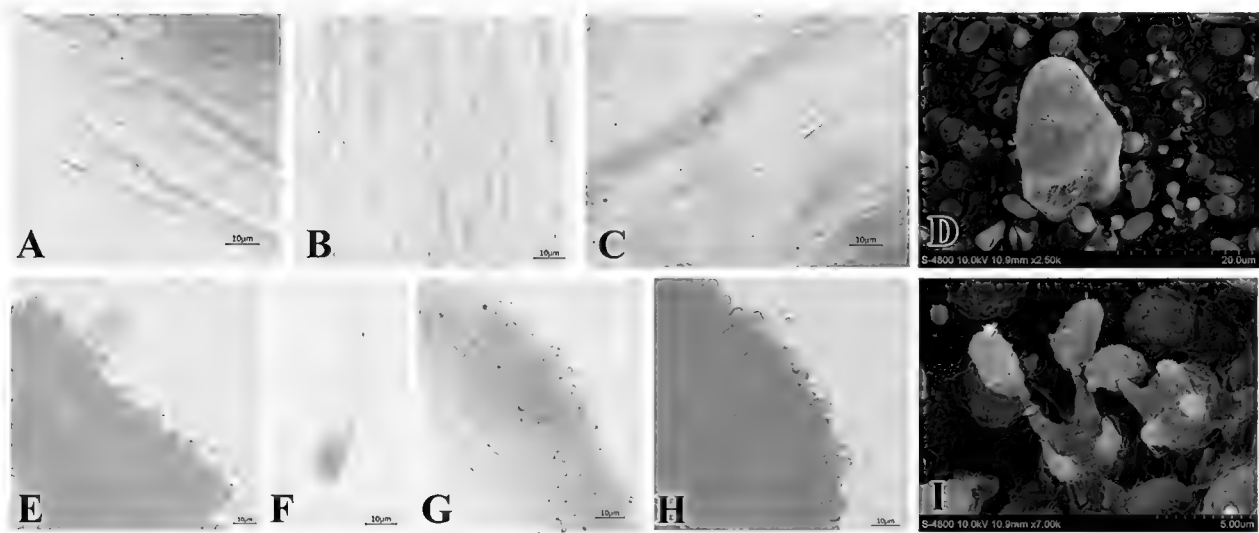FIG. 1. *Mycena cinereoalba* (holotype, QHU20461): basidiomata in the field.

FIG. 2. *Mycena cinereoalba* (holotype, QHU20461): A. pileipellis; B. stipitipellis; C. stipititrama; D, F. cystidia; E. cystidia and basidia; G–I. basidia, stipititrama, and basidiospores. Scale bars = 10 μm.

Basidiospores 6.25–11.25 × 2.5–3.75 μm, Q = 1.67–3.0, Qm = 2.23. long ellipsoid or ellipsoid to cylindrical, amyloid or weakly amyloid, smooth, hyaline, thin-walled, some with oily contents. Basidia 25.0–32.5 × 5.0–7.5 μm, clavate, smooth, colorless, 2–4 spored. Cheilocystidia 52.5–65.0 × 12.5–18.75 μm, clavate and subcylindric. Stipititrama nearly parallel. Pileipellis 18.75–25.0 μm wide irregular. Stipitipellis 5.0-20.0 μm wide nearly parallel. Clamp connections presented on all hyphae.

Habit and habitat: Scattered or grouped in conifer forest.

Additional specimens examined: **CHINA, Qinghai Province, Sanjiangyuan region**, Dongzhong forest district, 32.5039°N 97.6011°E, alt. 3850 m, 18 Aug 2019, L.C. Bai & T.T. Qiang (Holotype, QHU19358).

Based on molecular information combined with distinctive morphological data, this is proposed as a new species of *Mycena* from China.

Phylogeny

ITS analysis   Fig. 3

The ITS dataset included 41 sequences from 18 *Mycena* species and one *Maramius siccus* outgroup. Through BLASTn analysis, the ITS sequence of the new species (OK091026, length 663 bp) was 89.49% similar to a Chinese collection of *Mycena pura* (MW855913), with a 99% query coverage. In the ITS phylogram, *Mycena cinereoalba* (OK091026) clustered in a branch with *M. pura* (MW855913), and was a sister species of *Mycena seminau* (KF537250, NR154170), *Mycena sinar* (KF537249, NR154169), and *Mycena sinar* var. *tangkaisinar* (KF537251, NR154171).

nrLSU analysis   Fig. 4

The nrLSU dataset included 32 sequences from 19 *Mycena* species and one *Maramius siccus* outgroup. The nrLSU sequence of the new species (OK091029, length 1050 bp) was 98.74% similar to a Chinese collection of *Mycena* cf. *pura* (LC164935), with a 97% query coverage. In the nrLSU phylogram, *M. cinereoalba* (OK091029) clustered in a branch with *Mycena* cf. *pura* (LC164935), and was a sister species of *Mycena rosea* (MK278406).

Discussion

Mycena cinereoalba is well characterized by its grayish-white pileus, with stripes at the edge, context is thin and white, its lamellae are gray and wrinkled on the surface, with a transverse vein between them. The phylogenetic tree inferred from the ITS sequences showed that *M. cinereoalba* clustered with *Mycena pura* (MW855913). The colors of *M. pura* are variable, the pileus is typically lilac to purple in color when young. The lamellae are attached to the stem by a tooth and are white or slightly pink to purple in color, developing
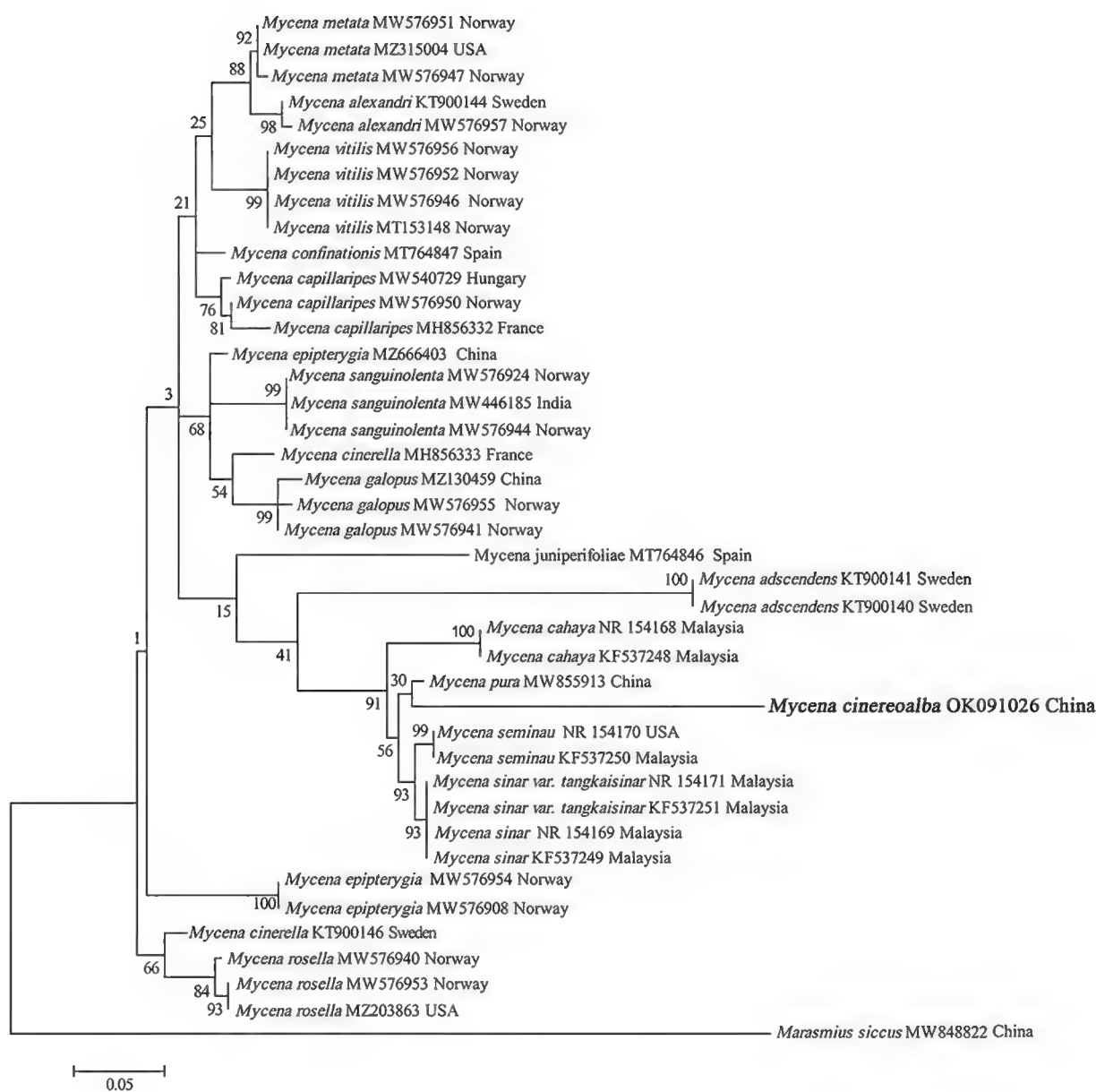
Fig. 3. Phylogenetic relationships of *Mycena* species inferred from ITS sequences using Maximum Likelihood, with *Marasmius siccus* as the outgroup. Bootstrap support is indicated at nodes. The newly described species is indicated in bold.

cross-veins when matured. The stipe is smooth (or has tiny hairs) on the surface. It tastes radish and growing on forest debris under hardwoods and conifers, growing in spring, summer and fall. The spores are long-elliptical or nearly cylindrical. The cheilocystidia and pleurocystidia are scattered or abundant, and fusoid-ventricose to widely fusiform (Kibby 2017).

The phylogenetic tree inferred from the nrLSU sequences showed that *M. cinereoalba* clustered with that of *Mycena* cf. *pura* (LC164935). The microscopic characteristics of *Mycena* cf. *pura* and *M. pura* are similar (as implied by their names). *Mycena cinereoalba* is a sister species to *M. rosea*. The pileus of *M. rosea* has a light pink to pink hue, and its edge has short stripes. It smells strongly
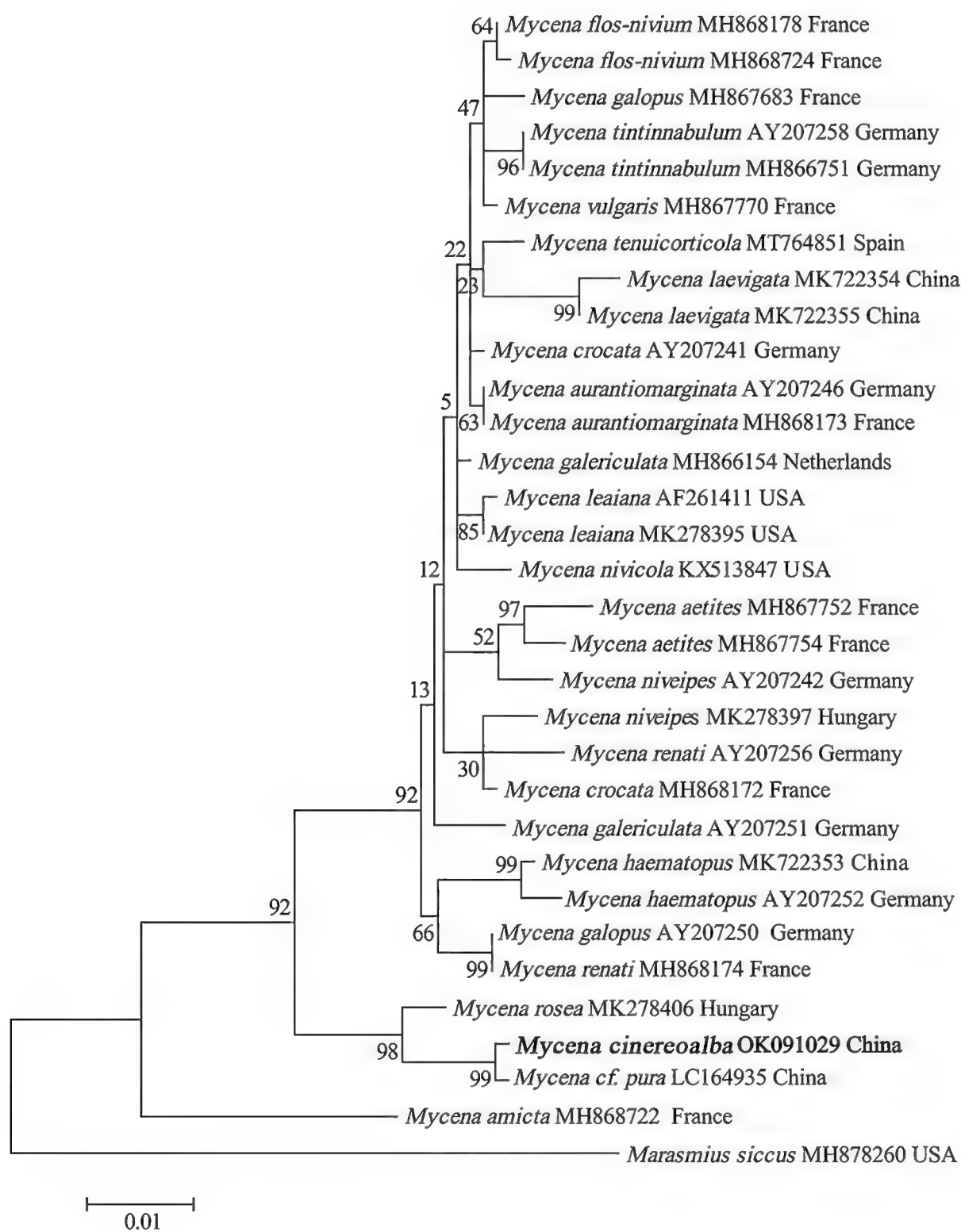
Fig. 4. Phylogenetic relationships of *Mycena* species inferred from nrLSU sequences using Maximum Likelihood, with *Marasmius siccus* as the outgroup. Bootstrap support is indicated at nodes. The newly described species is indicated in bold.

raphanoid. The base of its stipe is normally clavate. Its spores are amyloid, cheilocystidia and pleurocystidia were present, and clamp connections were observed. It is widely reported in Britain, especially in the southern counties of England.

By investigating and combining the diversity of macrofungi in the Jiangxi Forest Farm in Qinghai Province, this study provides important theoretical support for the study of *Mycena* in China and is beneficial to the conservation and utilization of macrofungal germplasm resources.

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Neochrosporium camelliae-sinensis gen. & sp. nov. from tea in China

Li-Yun Liang[1], Rui-Ping Liu[1], Qing-Zhou Ma[2], Jia-Yuan Cao[2],
Ya-Shuang Guo[2], Yue-Hua Geng[2*], Meng Zhang[2*]

[1] *College of Horticulture, Henan Agricultural University, Zhengzhou, Henan 450000, China*
[2] *College of Plant Protection, Henan Agricultural University, Zhengzhou, Henan 450000, China*
* Correspondence to: *gengyuehua@163.com, zm2006@126.com*

Abstract —A new hyphomycete genus and species, *Neochrosporium camelliae-sinensis*, was isolated from the leaves of Xinyang Maojian tea. Morphological and molecular (ITS, LSU) analyses indicated that the strain represented a new genus *Neochrosporium* with the type of *Neochrosporium camelliae-sinensis*. The distinct morphological features are as follows: white PDA colonies, 0-septate phialidic conidia, and absence of ramoconidia.

Key words —phylogenetic analysis, hyphomycete, *Pleosporales, Theaceae*

Introduction

Tea is a beverage made from *Camellia sinensis* (L.) Kuntze (*Theaceae*). Tea plants are hardy evergreen shrubs or small trees and are one of the most important economic crops in China. Tea drinking has a long history in China. In recent years, there have been a number of studies on tea plant microorganisms, the microbial species contained in them are important for the study of tea plant microorganisms as well as microbial control (Lu & al. 2007).

Cladosporium-like hyphomycetes are widespread in the world, including endophytic, phytopathogenic, and saprobic species (Crous & al. 2007). The ITS and LSU sequences have been used to re-evaluate *Cladosporium*-like taxa (Braun & al. 2003; Crous & al. 2007). *Ochrocladosporium* is one genus of *Cladosporium*-like fungi with ramoconidia (Pereira & al. 2022).

Ochrocladosporium was erected by Crous & al. (2007) with the type species *O. elatum* (Harz) Crous & U. Braun and is characterized by mycelium composed of branching septate hyphae, conidia solitary, erect, subelliptical. Colonies on PDA are rich in hyphae with a large number of production bags and uniform edges, ramoconidia present, and conidia occurring in branched chains (Crous & al. 2007). In this study, a new endophytic strain was obtained from fresh tea leaf, with sequences of ITS and LSU very similar to *Ochrocladosporium elatum*, but ramoconidia absent. The strain is proposed here as *Neochrosporium camelliae-sinensis*, in a new monotypic genus.

Materials and methods

Isolation

Tea tissue samples in polyethylene bags (Singh & Kamal 2012) were removed and washed with sterile water. They were cultured on PDA medium by tissue isolation method to isolate and purify the fungus in an incubator at 25°C for 7–15 days. Fungi were mounted in a drop of lactophenol oil on microscope slides. Species identification was performed based on morphological characteristics of the fungi (Fu & al. 2019) using a Nikon N2 optical microscope (Nikon Y-IDT, JAPAN). The holotype and ex-type culture are deposited in the Fungal Herbarium, Henan Agricultural University, Zhengzhou, China (HHAUFF).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh mycelium using CTAB method (Doyle 1991). Primers ITS1 and ITS4 (White & al. 1990) were used to amplify the rDNA ITS region; LR5 and LROR (Stielow & al. 2015) were used for LSU region (Fernández & al. 1999). The PCR mixture was amplified using an Eppendorf MasterCycle gradient thermal cycler (Eppendorf, Hamburg, Germany) with the procedure: initial denaturation at 94°C for 5 min; 35 cycles of 45 s at 94°C, 30 s at 55°C, and 90 s at 72°C; final extension at 72°C for 10 min, and incubation at 4°C. PCR products were detected on a 1% agarose gel, and sequences were obtained on an ABI 3730XL automated DNA Analyzer at Sangon Biotech (Shanghai) Co. Ltd.

Sequence alignment and phylogenetic analyses

The bidirectional sequences obtained from LSU were spliced by using DNAMAN software (Liu & al. 2011), and the spliced sequences were assembled with the sequences from ITS. The sequences were blasted in GenBank (Sayers & al. 2022), and related sequences were downloaded and included in the analysis (Table 1). The sequences were manually aligned and edited using MEGA7 (Kumar & al. 2016) and the trees were built by MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003) with 10 million generations bootstrap. The run was ended when the mean standard deviation of the split frequency was below 0.01.

TABLE 1. Species and sequences used in the phylogenetic analyses. New sequences are in bold.

FAMILY	SPECIES NAME	STRAIN	GenBank no.	
			ITS	LSU
Stachybotryaceae	*Alfaria acaciae*	CPC 31940	MH107882	MH107930
Pleosporales, incert.	*Camarosporidiella elaeagnicola*	MFLU15-1924	MF434135	MF434223
Didymellaceae	*Cladosporium bruhnei*	CBS 723.79	EU167558	KC800751
Didymellaceae	*Cladosporium* sp.	AIT_F1186	MF276663	MF276663
Leptosphaeriaceae	*Coniothyrium wernsdorffiae*	CBS 150.34	MH855474	MH855474
Dothioraceae	*Dothiora pyrenophora*	CPC 30634	KY929146	KY929179
Pleosporales, incert.	*Foliophoma fallens*	CBS 284.70	KY929148	GU238078
Pleosporales, incert.	**Neochrosporium camelliae-sinensis**	**HHAUF220225**	**OP605503**	**OP623515**
Cucurbitariaceae	*Neocucurbitaria rhamni*	C133	MF795777	MF795777
Cucurbitariaceae	*Neocucurbitaria rhamni*	C190	MF795778	MF795778
Cucurbitariaceae	*Neocucurbitaria rhamni*	C277	MF795779	MF795779
Pleosporales, incert.	*Ochrocladosporium elatum*	CBS 146.33	EU040233	EU040233
Pleosporales, incer.	*Ochrocladosporium frigidarii*	CBS 103.81	EU040234	EU040234
Pleosporales, incert.	*Parapleurotheciopsis inaequiseptata*	MUCL 41089	EU040235	EU040235
Thyridariaceae	*Parathyridaria philadelphi*	CBS 143432	MH107905	NG059873
Thyridariaceae	*Parathyridaria robiniae*	MFLUCC 14-1119	NR168161	KY511141
Phaeosphaeriaceae	*Phaeosphaeria avenaria*	AFTOL-ID 280	AY544725	AY544684
Cladosporiaceae	*Rachicladosporium luculiae*	CPC 11407	EU040237	EU040237
Didymellaceae	*Stagonosporopsis caricae*	HEYB4	MZ145054	MZ145055

Phylogenetic results

The final data set included information from 18 strains. The phylogenetic tree (FIG. 1) indicates that our *Neochrosporium* strain clustered with the two *Ochrocladosporium* strains (*O. elatum* and *O. frigidarii*); and this clade was sister group of *Necucurbitaria* and *Parathyridaria* strains.
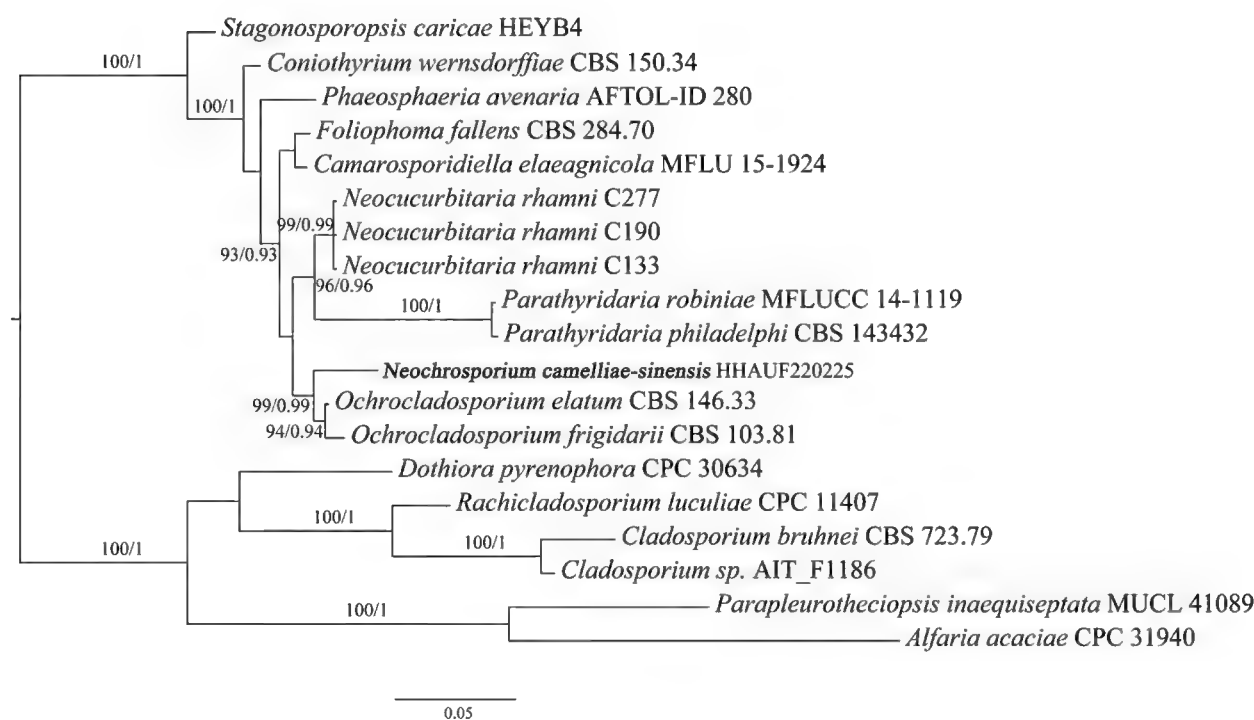
```
        ┌── Stagonosporopsis caricae HEYB4
100/1   │  ┌─ Coniothyrium wernsdorffiae CBS 150.34
        │  │  ┌── Phaeosphaeria avenaria AFTOL-ID 280
        100/1 │  ┌ Foliophoma fallens CBS 284.70
              │  └ Camarosporidiella elaeagnicola MFLU 15-1924
                  ┌ Neocucurbitaria rhamni C277
          99/0.99 │ Neocucurbitaria rhamni C190
     93/0.93       │ Neocucurbitaria rhamni C133
          96/0.96       ┌ Parathyridaria robiniae MFLUCC 14-1119
                  100/1  └ Parathyridaria philadelphi CBS 143432
                  ┌── Neochrosporium camelliae-sinensis HHAUF220225
          99/0.99 ┌ Ochrocladosporium elatum CBS 146.33
          94/0.94 └ Ochrocladosporium frigidarii CBS 103.81
              ┌─── Dothiora pyrenophora CPC 30634
              │ 100/1 ┌─ Rachicladosporium luculiae CPC 11407
    100/1     │       │ 100/1 ┌── Cladosporium bruhnei CBS 723.79
              │       │       └ Cladosporium sp. AIT_F1186
              └── 100/1 ┌──── Parapleurotheciopsis inaequiseptata MUCL 41089
                        └──── Alfaria acaciae CPC 31940
```

0.05

FIG. 1. Phylogenetic tree for *Neochrosporium camelliae-sinensis* and similar *Cladosporium*-like species, inferred from combined ITS and LSU sequence alignment. Branches are labeled with maximum-likelihood bootstrap support ≥90% and Bayesian posterior probability ≥0.9. New sequence indicated in bold.

Taxonomy

Neochrosporium R.P. Liu, Meng Zhang & Y.H. Geng, **gen. nov.**
MB846038

> Differs from *Ochrocladosporium* by its white PDA colonies, its 0-septate phialidic conidia, and its lack of ramoconidia.

> TYPE SPECIES: *Neochrosporium camelliae-sinensis* R.P. Liu & al.

> ETYMOLOGY: related to the genus of *Ochrocladosporium*.

MYCELIUM consisting of brached, smooth, hyaline hyphae. Differs from *Ochrocladosporium* by its white colonies in PDA, its 0-septate phialidic conidia, and its lack of ramoconidia. COLONIES in shades of brown; the back of the colonies were slightly white. CONIDIOPHORES micronematous. CONIDIOGENOUS CELLS monophialidic integrated, subcylindrical to doliiform, pale brown, smooth; determinate to sympodial, loci conically truncate, subdenticulate. CONIDIA 0-septate, oval, hyaline to pale brown, thin-walled, smooth to verruculose. Teleomorph unkown.

Neochrosporium camelliae-sinensis R.P. Liu, Meng Zhang & Y.H. Geng,
sp. nov. Fig. 2

MB846039

Differs from all related *Cladosporium*-like hyphomycetes by its white PDA colonies, and its lack of ramoconidia.

Type: China, Henan Province, Xinyang, *Camellia sinensis* leaf, 26 September 2021, R.P. Liu (**Holotype**, HHAUF220225; ex-type culture, HHAUF220225; GenBank OP605503, OP623515)
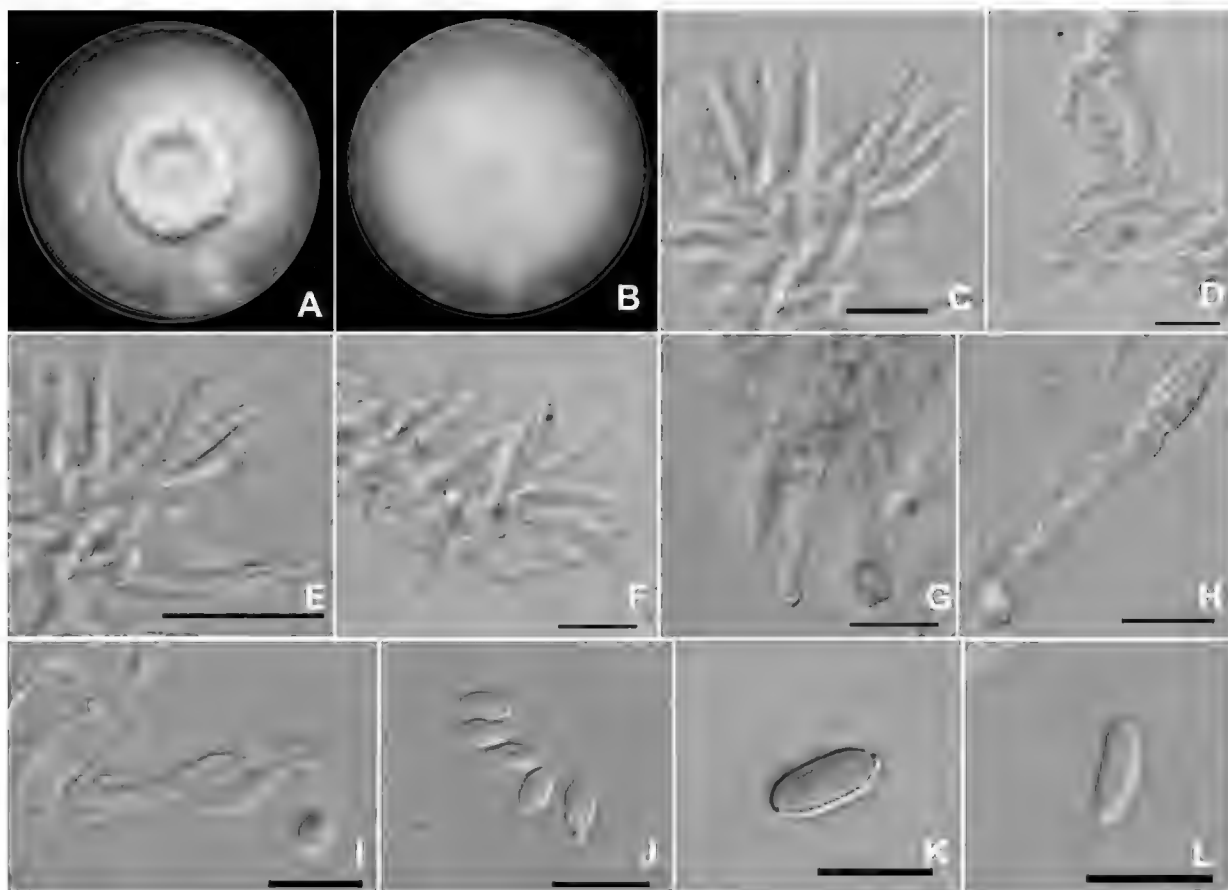
Etymology: named after the host *Camellia sinensis*.

Fig. 2. *Neochrosporium camelliae-sinensis* (holotype, HHAUF220225). A,.B. Colony; C–I. Conidiophores and conidiogenous cells; J–L. Conidia. Scale bars = 20 μm.

Colony on PDA at 25°C for 7 days reached to 6 cm in diameter, white, velvet or powdery, middle mycelium fluffy, the peripheral mycelium was longer fluffy. Mycelium mostly superficial, hyphae smooth, septate, hyaline, 1.0–3.5 μm. Conidiophores micronematous, mononematous, smooth, hyaline to pale brown, 0–1–septate, 3–5 phialides clustered together, usually branched, 15.0–25.5 × 1.5–4.5 μm. Conidiogenous cells monoblastic, discrete, subcylindrical to doliiform, branches, smooth, hyaline. Conidia 0–septate, round or long oval, smooth, thin–walled, hyaline, 5–10 × 2.5–3 μm. Telomorph unkown.

Comments — In the phylogeny, the *Neochrosporium* strain clustered with *Ochrocladosporium* strains (CBS146.33 and CBS 103.81) with 99% bootstrap. Molecular data for *Neochrosporium* and *Ochrocladosporium* are similar, 98% identity between *N. camelliae-sinensis* and *O. elatum*, and they clustered nearest in the phylogenetic tree. *Ochrocladosporium frigidarii* was isolated from cooled room in Germany (Crous & al. 2007), and has 99.5% identity with *O. elatum* in ITS and LSU .

In morphology, *Neochrosporium* can be clearly discriminated from *Ochrocladosporium*. *Ochrocladosporium* colonies are composed of branched and septate mycelium, which produce two kinds of conidia, with the commonly occurring ramoconidia (Crous & al. 2007). The conidia are arranged in chains. But in *Neochrosporium camelliae-sinensis*, colony is white, and ramoconidia absent and the conidia were formed on phialide. Although *Neochrosporium* and *Ochrocladosporium* are very close in phylogeny, but both of ITS and LSU are conserved in fungi (Schoch & al. 2012). The morphology and conidial forms of *Neochrosporium* and *Ochrocladosporium* are relatively different; and as these characters can be effective for diffentiating hyphomycete genera, *Neochrosporium* is supported as a new monotypic genus.

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Are *Tricholomopsis* spp. common or rare? – new data from Greece

Marina Triantafyllou, Ioanna Pyrri, Aristeidis Parmakelis, Zacharoula Gonou-Zagou*

Section of Ecology & Systematics, Department of Biology, National & Kapodistrian University of Athens, Panepistimiopolis, GR-15784 Athens, Greece

Correspondence to: zgonou@biol.uoa.gr

Abstract—This study reports the presence of *Tricholomopsis flammula* and *T. sulfureoides* in Greece, providing additional data on the morphology, phylogeny, distribution, and rarity of these species.

Key words—*Agaricales,* macromycetes, wood-inhabiting fungi, molecular analysis

Introduction

Tricholomopsis Singer comprises saprotrophic and mostly wood-inhabiting species with tricholomatoid fruitbodies that typically possess yellow color overall and fibrils or squamules on pileus surface that may be differently colored. The basidiospores are smooth, non-amyloid and white in deposit, and the large cheilocystidia form a sterile lamellae edge. It is distributed mainly in the Northern Hemisphere (Smith 1960; Holec 2012a; Hosen & al. 2020). The genus includes c. 50 names worldwide (http://www. indexfungorum.org). In Europe, *Tricholomopsis* is represented by a small number of species, while in literature, until quite recently, there were contradictory opinions about the number and identity of the species. Studies that combine both traditional morphological and molecular methods have confirmed the presence of six species in Europe, *T. rutilans* (Schaeff.) Singer, *T. decora* (Fr.) Singer, *T. flammula, T. sulfureoides, T. pteridiicola* Olariaga & al., and *T. badinensis* Holec & al. (Holec & Kolařík 2011, 2012; Olariaga & al. 2015; Holec & al. 2019). *Tricholomopsis rutilans* and

T. decora are characterized as common species in Europe, whereas *T. flammula, T. sulfureoides, T. pteridiicola,* and *T. badinensis* are considered rare with few recordings. The first finding of *T. sulfureoides* in Europe came from Estonia and was initially described as a new species, *T. osiliensis* (Vauras 2009). Three years later, Holec (2012b) reported the presence of the same species in Slovakia. Further studies showed that *T. osiliensis* was conspecific with *T. sulfureoides,* a species already known from N. America (Vauras & al. 2012). Saar & Voitk (2015) confirmed this synonymy by studying the type collection of *T. sulfureoides.* Holec & al. (2019) documented the existence of *T. sulfureoides* in Poland as well. *Tricholomopsis flammula,* which is also considered a rare species, has a wide distribution in Europe and it is also found in Pakistan (Holec 2009; Holec & Kolařík 2011, 2012; Razaq & al. 2012). *Tricholomopsis pteridiicola* is a recently described new species collected from Spain and France and associated with the fern *Pteridium aquilinum,* in contrast with all other European members of the genus which grow, like most species of *Tricholomopsis,* on conifer wood (Olariaga & al. 2015). *Tricholomopsis badinensis* is a newly described species, known until now only from Slovakia (Holec & al. 2019). *Tricholomopsis badinensis* along with *T. decora* and *T. sulfureoides* have in common the absence of red or violet coloration on the basidiomata.

Recent collections as well as old represented by dried specimens preserved unidentified in the Mycetotheca ATHUM were determined as the rare species *T. flammula* and *T. sulfureoides.* The aim of this study is to communicate the presence of *T. flammula* and *T. sulfureoides* in Greece, to confirm the variability of the species, and to add data on their morphology, phylogeny, distribution, and rarity.

Materials and methods

Morphological study

The studied material includes six collections of *T. flammula* and three of *T. sulfureoides* from different regions of Greece. Some of them were newly collected during the period 2012–15 from three National Parks of Greece (Mt. Ainos, Mt. Oiti, and Mt. Parnassos), while the rest were dried specimens preserved at the Dried Specimen Collection of the Mycetotheca ATHUM, National & Kapodistrian University of Athens. For comparison purposes, six collections of *T. rutilans* were studied additionally.

Macromorphological descriptions of *T. flammula* and *T. sulfureoides* are based on detailed observation of the newly collected specimens, as well as on descriptions accompanying the dried specimens. Colors were described in daylight conditions and color codes refer to Kornerup & Wanscher (1981).

Microscopic observations were made from dried material using a Zeiss AxioImager A1 Differential Interference Contrast (DIC) microscope. Sections were mounted in Melzer's reagent for observation, measurements and photography. Spores were measured in side-view (25 spores per specimen). Spore sizes are presented as a central range, calculated as: mean ± 2 × SD, flanked by parenthetical extreme values lying outside the range, and followed by mean length (L_m) and width (W_m). The length/width quotient (Q) is presented as the mean of all quotients, flanked by the minimum and maximum individual quotients.

Terminology used for description of macromorphological and micromorphological characteristics is in accordance with Vellinga (1988).

All specimens are deposited at the Dried Specimen Collection of the Mycetotheca ATHUM, National & Kapodistrian University of Athens, Greece.

Molecular analysis

Total genomic DNA was extracted from basidiocarps following the CTAB protocol of Winnepenninckx & al. (1993) as modified by Parmakelis & al (2003). Pieces from the flesh and lamellae of the pilei were placed in 700 ml 2× CTAB containing 20 μL proteinase-K (10 mg/ml). The mixture was incubated overnight in a thermoshaker at 840 rpm and 56°C. Proteins were removed with chloroform alone. DNA was precipitated with ice-cold absolute ethanol, washed with 70% ice-cold ethanol and suspended with 50 μl nanopure water.

The Internal Transcribed Spacer 1 and 2 with the intervening 5.8S ncrDNA (ITS region) was amplified with the oligonucleotide primer sets 18S-ITS1/28S-ITS2 (Pantou & al. 2005). PCR was performed in a 25 μl volume, in which 1 μl of template DNA was mixed with 0.2 mM dNTPs, 0.4 mM of each primer and 0.5 units of Taq polymerase. The concentration of the $MgCl_2$ was 3.5 mM. The target loci were amplified with a cycle program comprising an initial denaturation step at 95°C for 5min, followed by 35 cycles of 30 sec at 94°C, 45 sec at 48°C, and 2 min at 72°C, and ending with 7 min sequence extension at 72°C. Automated sequencing of both strands of the PCR amplicons was performed in a PE-ABI3740 automated sequencer (using Big-Dye terminator chemistry). The primers in the sequencing reactions were the same as in the PCR amplifications. All fragments were read in both directions. Consensus sequences were computed from forward and reverse sequences using Codon-Code Aligner (v. 4.1.1, Codon Code Corporation) and were deposited in the GenBank database under accession numbers from MT981245 to MT981251 (Table 1).

The authenticity of the DNA sequences and the homology with the target genetic locus were evaluated by a BLAST search in the NCBI genetic database (http://blast.ncbi. nlm.nih.gov/Blast.cgi). The generated *Tricholomopsis* sequences, along with reference sequences obtained from NCBI and UNITE databases (Table 1) were assembled and aligned using Codon-Code Aligner (v. 4.1.1, Codon Code Corporation).

Phylogenetic trees based on ITS were constructed using Neighbor Joining (NJ) with MEGA 10.0 (Kumar & al. 2016), Maximum Likelihood (ML) using RAxML-HPC2 on XSEDE 8.2.10 (Stamatakis 2014) through the CIPRES Science Gateway V.3.3 (Miller & al. 2010) and Bayesian Inference (BI) with MrBayes v. 3.2.7 (Ronquist & al. 2012).

TABLE 1. Specimens and sequences used inthe phylogenetic analysis. New sequences are given in bold. [HT]= holotype; [ST] = syntype.

Species	Voucher	GenBank (ITS)	Country	Reference
T. aurea	SFSU: DED 8327	MF100960	Africa	Desjardin & Perry 2017
	SFSU: BAP 618	MF100961	Africa	Desjardin & Perry 2017
T. badinensis	PRM 946195	LS992163	Slovakia	Holec & al. 2019
	PRM 899423	LS992164	Slovakia	Holec & al. 2019
	PRM 946194	LS992165	Slovakia	Holec & al. 2019
T. decora	UBC F23918	KJ146733	Canada	Berbee & al. (unpublished)
	TUF 106634	UDB011838	Estonia	Saar & Voitk 2015
	UBC F23917	KJ146732	Canada	Berbee & al. (unpublished)
	PRM 899160	HE649942	Slovakia	Holec & Kolařík 2012
	TENN 021494	KP453708	USA	Sanchez-Garcia (unpublished)
T. decora [as *T. sulphureoides*]	NYSf 3116.A[HT]	UDB018405	USA	Saar & Voitk 2015
T. flammula	S-F-156625	KP058975	Sweden	Olariaga & al. 2015
	SR 161	FR822742	Pakistan	Razaq & al. 2012
	ARAN-Fungi 00322	KP058973	Spain	Olariaga & al. 2015
	WU 25091	FN554892	Austria	Holec 2009
	PRM 909608	FN554896	Czech Repub.	Holec & Kolařík 2011
	WU 12087	FN554897	Austria	Holec & Kolařík 2011
	TUF 118261	UDB015434	Estonia	Saar & Voitk 2015
	PRM 899459	FN554894	Czech Repub.	Holec & Kolařík 2011
	PRM 899162	HE649939	Slovakia	Holec & Kolařík 2012
	PRM 899190	HE649941	Slovakia	Holec & Kolařík 2012
	PRM 899180	HE649940	Slovakia	Holec & Kolařík 2011
	WU 13075	HE652866	Austria	Holec & Kolařík 2012
	PRM 899108	FN554893	Czech Repub.	Holec 2009, Holec & Kolařík 2011
	ATHUM 9529	**MT981245**	**Greece**	**This study**
	ATHUM 9528	**MT981246**	**Greece**	**This study**
T. flavescens	NYSf 1195.1 [ST]	UDB022699	USA	Saar & Voitk 2015

Species	Voucher	GenBank (ITS)	Country	Reference
T. ornaticeps	PDD 82501	KY010820	New Zealand	Cooper & Park 2016
	PDD 102517	KY010822	New Zealand	Cooper & Park 2016
	PDD 102769	KY010824	New Zealand	Cooper & Park 2016
T. pteridiicola	ARAN-Fungi 00121	KP058988	Spain	Olariaga & al. 2015
	ARAN-Fungi 00321	KP058992	France	Olariaga & al. 2015
	ARAN-Fungi 00122	KP058990	Spain	Olariaga & al. 2015
T. rutilans	PRM 889120	FN554895	Slovakia	Holec 2009
	BIO-Fungi 11313	KP058979	Sweden	Olariaga & al. 2015
	H 6012126	UDB031626	Finland	FinBOL project (unpublished)
	PRM 899460	HE649946	Czech Republic	Holec & Kolařík 2012
	TUF 106244	UDB011443	Estonia	Saar & Voitk 2015
	UBC F16251	EF530929	Canada	Denis & al. (unpublished)
	ATHUM 7713	**MT981249**	**Greece**	**This study**
	ATHUM 7714	**MT981250**	**Greece**	**This study**
	ATHUM 9524	**MT981251**	**Greece**	**This study**
T. rutilans var. *splendidissima*	LIP 86127	KP058995	France	Olariaga & al. 2015
T. aff. *rutilans*	ARAN-Fungi 00323	KP058982	Spain	Olariaga & al. 2015
	UPS F- 646220	KP058984	Sweden	Olariaga & al. 2015
	TUB 011582	KP058981	Germany	Olariaga & al. 2015
T. scabra	PDD 102100	KY010821	New Zealand	Cooper & Park 2016
	PDD 102579 [HT]	KY010823	New Zealand	Cooper & Park 2016
T. sulfureoides	NYSf 3116.10 [HT]	UDB023125	USA	Saar & Voitk 2015
	PRM 945185	LT984728	Poland	Kolařík (direct submission)
	ATHUM 9527	**MT981247**	**Greece**	**This study**
	ATHUM 9526	**MT981248**	**Greece**	**This study**
T. sulfureoides [as *T. osiliensis*]	TUF 101571	UDB015070	Estonia	Saar & Voitk 2015
	TUF 101571	HE649944	Estonia	Holec & Kolařík 2012
Pluteus romellii	LE 217944	FJ774073	Russia	Malysheva & al. 2009

NJ analysis was conducted with a heuristic search with the following settings. The substitution model was Kimura 2 parameter with substitutions including transitions and transversions. Bootstrap support values were derived from 1000 replications and are indicated at the branching nodes. For ML the General Time Reversible (GTR) substitution model with gamma distribution and invariable sites was used and bootstrap supports were estimated by 1000 replicates. A Markov Chain Monte Carlo (MCMC) algorithm was used to generate the phylogenetic tree for the ITS genetic locus. The GTR substitution model with gamma distribution and invariable sites was chosen after testing in MEGA. The analysis was run in duplicate with four MCMC chains for 10,000,000 generations and stopped when the average standard deviation of split frequencies reached below 0.01. Random trees were sampled every 100 generations. Burnin was set to 25% after which the likelihood values were stationary. The species *Pluteus romellii* FJ774073 of the related family *Pluteaceae* was used to root the tree. The resulting trees were viewed with TreeView v. 1.6.6 and, together with the alignments, deposited into TreeBASE (http://www.treebase.org) under submission ID 27845.

Results

Morphological study

Tricholomopsis flammula Métrod ex Holec, J. Nat. Mus. (Prague), Nat. Hist. Ser.
178: 8 (2009)   Fig. 1

Pileus 30–60 mm, first hemispherical to convex, at maturity plano-convex with a broad umbo, center sometimes depressed, surface pale yellow (2A3, 3A3), light yellow (2A5) to pastel yellow (3A4), covered with tiny greyish magenta (13E5, 14E5), greyish violet (18E6–7, 18D6, 16E6–7, 16D5, 15E6–7, 15D5–6) to dark purple (14F5–6) scales, in young fruitbodies densely arranged (sometimes covering the whole surface), in mature fruitbodies densely arranged at center, loosely arranged towards the margin, sometimes scales reaching the margin more pale, reddish brown (8D4), brownish violet (11D6), greyish red (11D5), greyish ruby (12E5) to greyish magenta (13E5, 14E5), margin in young specimens mostly inflexed, in mature specimens inflexed or straight, often undulate and/or orange colored; lamellae adnate, crowded, yellow (3A6) to yellowish white (3A2); stipe 40–90 × 7–12 mm central, cylindric, sometimes tapering upwards, straight to slightly curved, surface smooth to fibrillose, white, in mature specimens at some places/ or after pressing pastel yellow (3A4), with scarce greyish violet fibrils near the apex in a mature specimen, while in the same specimen with obscure pale violet felty covering when young; context yellowish; basidiomes growing in small groups.
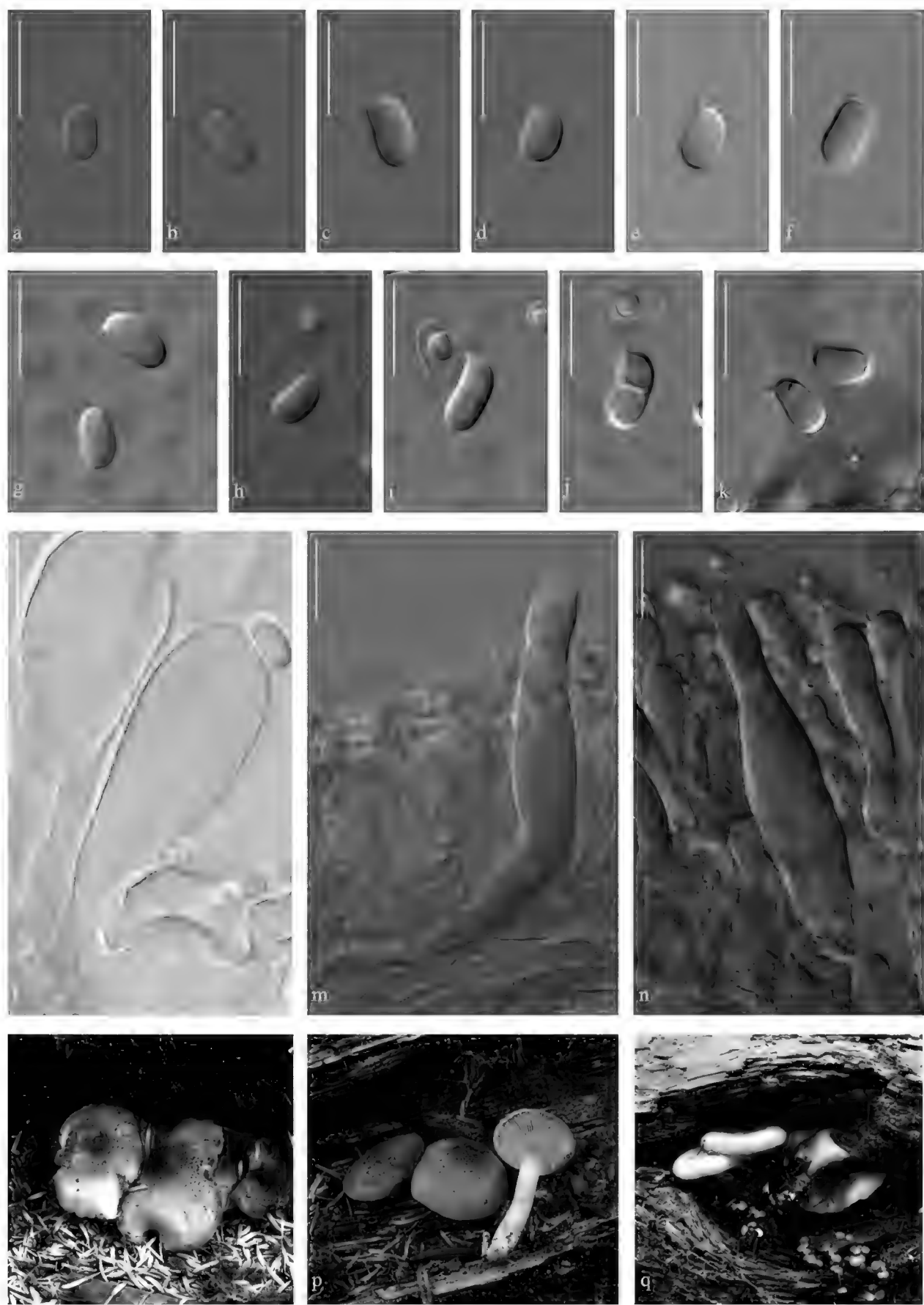
Fig. 1. *Tricholomopsis flammula*: a–k. Basidiospores [a, b. ATHUM 9529; c, d. ATHUM 9543; e, f. ATHUM 9545; g–k. ATHUM 9528]; l. Cheilocystidia [ATHUM 9546]; m, n. Pleurocystidia [m. ATHUM 9543; n. ATHUM 9528]; o–q. Basidiomes [o, p. ATHUM 9546; q. ATHUM 9529]. Scale bars = 10 μm.

BASIDIOSPORES (4.2–)4.9–8.1(–8.9) × (3–)3.1–4.2(–4.6) μm, L_m = 6.5 μm, W_m = 3.6 μm, Q = 1.32–1.79–2.47, ellipsoid to cylindrical, mostly oblong and obovoid, some phaseoliform, less often almost lacrymoid, smooth, hyaline, inamyloid, some with one guttule; BASIDIA 16–23 × 5–6 μm, clavate, narrowly clavate to cylindric, 4-spored; CHEILOCYSTIDIA (16–)25–60(–70) × (9–)11–21(–27) μm, forming a sterile edge, clavate, mostly broadly clavate; PLEUROCYSTIDIA (32–)38–60(–67) × (5–)6.5–9.5(–14.5) μm, abundant, irregularly cylindric, narrowly clavate, clavate, narrowly fusiform to fusiform, less often almost lageniform, utriform with tapering base, with yellow, golden-yellow, "oily" content; LAMELLAR TRAMA regular, hyphae 6–16 μm, some with yellow content; PILEIPELLIS a cutis of hyphae 5–10 μm wide, upper layer of hyphae with brownish-red intracellular granular pigment; CLAMP CONNECTIONS present.

HABITAT—natural, pure forests of *Abies cephalonica* Loudon, usually from 900–1500 m asl, on dead wood of *A. cephalonica*, mostly on fallen, decaying trunks.

SPECIMENS EXAMINED—**GREECE: ACHAIA, Mt. Klokos**, Natura 2000 protected area, on dead wood of *A. cephalonica*, 22.11.1999, leg. P. Tsopelas (ATHUM 9543); **ATTIKI, Mt. Parnitha National Park**, Mola, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 17.11.2004, leg. G. Athanasakis (ATHUM 9545); **FTHIOTIDA, Mt. Oiti National Park**, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 22.10.2013, leg. C. Chondralis (ATHUM 9528); **KEFALONIA ISLAND, Mt. Ainos National Park**, recreational park, 1470 m asl, pure forest of *A. cephalonica*, on fallen dead trunk of *A. cephalonica*, 12.10.2012, leg. M. Triantafyllou & Z. Gonou-Zagou (ATHUM 9546); recreational park, pure forest of *A. cephalonica*, 13.10.2013, leg. A. Tsasis & G. Tsasi (ATHUM 9547); Eza, 950 m asl, pure forest of *A. cephalonica*, on fallen dead trunk of *A. cephalonica*, 29.10.2012, leg. M. Triantafyllou (ATHUM 9529).

Tricholomopsis sulfureoides (Peck) Singer [as "*sulphureoides*"], Ann. Mycol. 41(1/3): 69 (1943)   FIG. 2

≡ *Agaricus sulfureoides* Peck, Ann. Rep. N.Y. State Mus. 23: 86 (1872)

= *Tricholomopsis osiliensis* Vauras, Folia Cryptog. Estonica 45: 87 (2009)

PILEUS 40–80 mm, convex to plano-convex, center depressed or with a broad umbo, surface smooth, greenish yellow (1A6), pastel yellow (1A4, 2A4), light yellow (2A5) to yellow (2A6), one fruitbody with greyish yellow (2B4) spots and grey brown tinges at umbo, pileus covered almost completely with fine, light yellow (3A5), greyish yellow (4C5) to golden (4C6) fibrils, margin mostly inflexed, often radially cracked, mature fruitbodies evolving orange, orange brown or grey brown spots (maybe after handling); LAMELLAE adnate sometimes with a decurrent tooth, medium crowded to crowded, pastel yellow
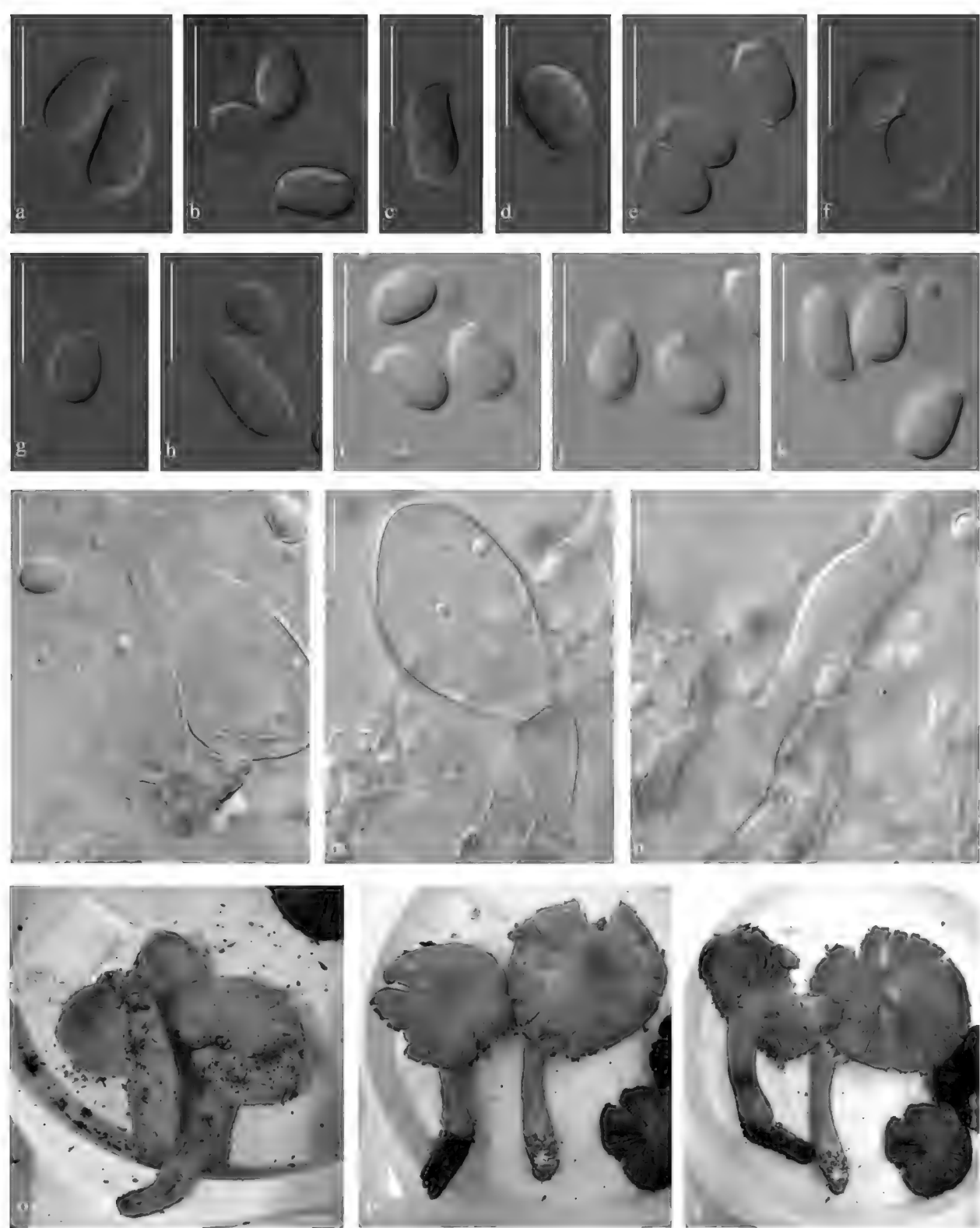
Fig. 2. *Tricholomopsis sulfureoides*: a–k. Basidiospores [a–e. ATHUM 9526; f–h. ATHUM 9548; i–k. ATHUM 9527]; l–n. Cheilocystidia [ATHUM 9527]; o–q. Basidiomes [o. ATHUM 9527; p, q. ATHUM 9548]. Scale bars = 10 μm.

(1A4, 3A4) with greenish yellow (1A6) or greyish yellow (2B4) tints; STIPE 50-70mm,cylindric, swollen at the median part or at base, slightly curved, almost concolorous with pileus, surface light yellow (1A5, 2A5), pastel yellow (1A4), yellow (2A6), greyish yellow (2B4), in one collection with scarce brown (5F4, 6F4) to dark brown (7F4–5) contrasting fibrils; CONTEXT yellowish; BASIDIOMES growing in small groups.

BASIDIOSPORES (5.7–)6.3–9.8(–11.4) × (3.7–)3.9–5.6(–6.1) μm, L_m = 8 μm, W_m = 4.8 μm, Q = 1.34–1.68–2.34, ellipsoid to cylindrical, mostly oblong and obovoid, a few phaseoliform, smooth, hyaline, inamyloid, some with one guttule; BASIDIA 32–40 × 5–6 μm, narrowly clavate to cylindric, 4-spored, sterigmata 3.5–5.5 μm long; CHEILOCYSTIDIA 22–75 × 12–26 μm, ovoid to obovoid, broadly fusiform to broadly clavate, mixed with a few cylindrical or irregularly lageniform, some with one (rarely two or more) projections, up to 15μm, at/near apex; PLEUROCYSTIDIA 40–65 × 5–6.5(–8) μm, frequent, irregularly cylindrical to cylindric-fusiform, often with constrictions, some with yellow content, few emerging deep from trama; LAMELLAR TRAMA regular; PILEIPELLIS a cutis of pale brown hyphae 3–7 μm broad; CLAMP CONNECTIONS present in all tissues.

HABITAT—natural, pure forests of *Abies cephalonica,* from 1400–1800 m asl, on dead wood of *A. cephalonica.*

SPECIMENS EXAMINED—**GREECE: ARCADIA, Mt. Mainalo**, Natura 2000 protected area, 1800 m asl, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 21.10.2001, leg. G. Prountzopoulos (ATHUM 9526); **FOKIDA-BOIOTIA, Mt. Parnassos National Park**, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 10.11.2012, leg. group of foray & Z. Gonou-Zagou (ATHUM 9527); Varkos Despoti, 1400 m asl, pure forest of *A. cephalonica*, on stump of *A. cephalonica*, 7.11.2015, leg. I. Pyrri & A. Sergentani (ATHUM 9548).

Phylogenetic analysis

Phylogenetic analysis of ten species of *Tricholomopsis* discloses four main clades, the two sister clades of *T. decora* and *T. badinensis* and two related but well-separated clades, the basal one comprising the species of southern hemisphere, while the other encompasses all remaining species. Greek specimens of *T. sulfureoides* cluster with other European and American *T. sulfureoides* specimens in a well-supported clade (FIG. 3). Concerning *T. flammula,* the phylogenetic tree reveals the same general topology as previous studies (Holec & Kolařík 2012; Olariaga & al. 2015). Therefore, the two sister subgroups that correspond to the "Western and Central European" and "Carpathian" clades of Holec & Kolařík (2012) are present as well (FIG. 3). The specimen from Pakistan is nested within the former clade. Interestingly, the

Fig. 3. Phylogenetic tree constructed from aligned DNA sequences of the ITS region as produced by ML. Sequences obtained during this study are presented in bold. Bootstrap values >70% for RAxML (numbers in bold) and NJ (numbers in italics), and posterior probabilities >0.95 for BI (numbers in bold underlined) are indicated at the nodes. *Pluteus romelli* LE 217944 was chosen as outgroup.

Greek specimen ATHUM 9529 is included in the "Carpathian" clade, whereas the Czech specimen PRM 899108 stands out of the two subclades forming a sister clade to them, and the Greek specimen ATHUM 9528 represents a basal clade to all.

Discussion

Tricholomopsis flammula

Tricholomopsis flammula was first described from France and is regarded as a rare species though it has a wide distribution in Europe and it is found also in Pakistan (Holec 2009; Holec & Kolařík 2011, 2012; Razaq & al. 2012). Most important characteristics of *T. flammula* that distinguish this species from the common *T. rutilans* are the yellowish stipe, typically without red or violet colors and the more ellipsoid spores, as it is also indicated by the Q values of spores for both species. The abundance of pleurocystidia in specimens of *T. flammula* was previously also used as a separating feature from *T. rutilans* (Krisai-Greilhuber & Voglmayr 2000; Holec 2009; Holec & Kolařík 2011). Olariaga & al. (2015) observed that pleurocystidia can be also found in specimens of the *T. rutilans* s.str. clade, indicating that this feature can no longer be highlighted when trying to separate *T. flammula* from *T. rutilans* s.str. Most Greek specimens of *T. rutilans* deposited at the Mycetotheca ATHUM possess no pleurocystidia, but in some specimens infrequent pleurocystidia are observed. Spores of Greek collections of *T. flammula* are rather narrow (4.9–8.1 × 3.1–4.2 μm, Q = 1.32–1.79–2.47) and comparing them with the spores of Greek collections of *T. rutilans* ((5.3–)5.8–8.3(–9) × (3.9–)4.1–5.6(–6) μm, Q = 1.2–1.46–1.87) it is confirmed that the more reliable distinguishing feature between the two species is the spore shape indicated by Q values.

Holec & Kolařík (2011, 2012) stated that there is molecular and morphological variation among specimens of *T. flammula*. In their study on the genus *Tricholomopsis* in Europe they noted that Slovak specimens of *T. flammula* possess more elongated spores than other European collections and develop scarce violet fibrils on stipe of mature basidiomes that can be more evident in young fruitbodies as a violet "felt". In addition, molecular analysis showed that the Slovak specimens together with one Czech collection (PRM 899459) grouped together in the phylogenetic tree and this clade was characterized as "Carpathian" *T. flammula* subpopulation. Our molecular analysis supports the two sister subclades, the "Western and Central European" and the "Carpathian", in the *flammula* clade. The specimen ATHUM 9529 is grouped within the "Carpathian" clade, whereas the ATHUM 9528 is a basal clade to

all specimens of the species. The Czech specimen PRM 899108 is excluded from the two subclades of the *flammula* clade forming a sister clade to it. The last two collections, when compared morphologically, reveal differences both in spore size and colors of the basidiomes, the Greek specimen having more elongated spores (4.4–)5.3–8.7(–8.9) × (3–)3.1–4.2(–4.6) μm vs (5.2–)5.5–6.4 × 3.2–4.0 μm) and densely arranged purple fibrillose scales mainly in the center of the pileus. They were not observed any 2-spored basidia that could justify the production of longer spores, as Holec & Kolařík (2011) mentioned for the Czech specimen PRM 899459. Spore size and shape vary slightly between the various Greek collections, as well as within the same collection. Nevertheless, all of them fit well in the "Carpathian" subclade because of the accordance of their mean Q = 1.79 with Holec & Kolařík's (2012) data (= 1.72–1.90).

It is worth mentioning that sample ATHUM 9528, which seems to deviate phylogenetically, does not show any important morphological difference from other typical *T. flammula* specimens, except having slightly more elongated spores (TABLE 2). In addition, specimen ATHUM 9547 is the only one similar to the Slovak specimens in terms of the presence of scarce violet fibrils on the apex of the stipe of a mature fruitbody and the obscure pale violet felty covering of a young fruitbody (Holec & Kolařík 2012). Inferentially, there is morphological and genetic infraspecific variability that might reveal an allopatric speciation, but cannot justify the description of new taxa yet, not until new data are presented.

TABLE 2. Mean spore sizes of Greek collections of *Tricholomopsis flammula*

Voucher	Vegetation	Locality	Length	Width	L/W
ATHUM 9546	*A. cephalonica*	Mt. Ainos	6.1	3.6	1.72
ATHUM 9529	*A. cephalonica*	Mt. Ainos	6	3.5	1.69
ATHUM 9547	*A. cephalonica*	Mt. Ainos	6.2	3.5	1.78
ATHUM 9528	*A. cephalonica*	Mt. Oiti	7	3.6	1.93
ATHUM 9543	*A. cephalonica*	Mt. Klokos	6.3	3.7	1.69
ATHUM 9545	*A. cephalonica*	Mt. Parnitha	6.8	3.8	1.77

In Europe, *T. flammula* grows mostly on decaying trunks of conifers (*Abies, Picea*), occasionally also on dead wood of broadleaf trees and does not seem to have a preference over protected areas (Holec & Kolařík 2011, 2012). All

specimens studied were found on dead wood of *Abies cephalonica,* in *A. cephalonica* forests of Western, Central Greece, and the Peloponnese, in sites inside National Parks and Natura 2000 protected areas, the protection status of the latter being disputable. It is worth mentioning, that *T. flammula* is found in Mt. Ainos in the island of Kefalonia, which is the type locality of the endemic species *A. cephalonica,* the forests of which represent a Mediterranean mountainous habitat quite different from the above-mentioned Central and North European habitats. *Tricholomopsis flammula* is so far recorded only from *A. cephalonica* forests in Greece, while the closely related *T. rutilans* has been found growing also on dead wood of *Abies borisii-regis.* The number of samples indicates that the species seems to be rare, though widespread within the distribution area of pure *A. cephalonica* forests, where its appearance is rather uncommon. It is noteworthy, that pure *A. cephalonica* forests occur mainly in Central Greece and Peloponnese. Additionally, the species is reported from *Fagus* forests in Mt. Vasilitsa and Mt. Grammos, in Northwestern Greece (Konstantinidis 2006), where it is characterized as unusual. We did not have the chance to study specimens from the broadleaf forests, if there are any. To our knowledge, the Greek collections of *T. flammula* reveal the southernmost distribution of the species in Europe.

Tricholomopsis sulfureoides

Tricholomopsis sulfureoides was originally described from North America and in Europe was first recorded in Estonia quite recently as *T. osiliensis* (Vauras 2009). The species was recorded twice as *T. osiliensis* from Estonia on fallen trunk of *Picea abies* (L.) H. Karst. (Vauras 2009; Kalamees 2011) and from a virgin forest in Slovakia on *Abies alba* Mill. (Holec 2012b). Holec & al. (2019) referred to the presence of *T. sulfureoides* in Poland in five sites from the Białowieża virgin forest on fallen trunks of *P. abies.* They reported also two more collections of the species, one from Norway and one from Moscow in Russia. Dovana & Galbusera (2019) reported the occurrence of *T. sulfureoides* in Northern Italy. Interestingly, *T. sulfureoides* was recently proposed for assessment at the Global Fungal Red List Initiative and was preliminarily assessed as vulnerable (VU). In the same List the occurrence of the species in another four European countries is mentioned (Spain, France, UK, Switzerland) without any further details.

In 2012, during an educational foray for biology students, two yellowish fruitbodies belonging to *Tricholomopsis* were collected from a protected fir (*A. cephalonica*) forest in Central Greece (Mt. Parnassos National Park). Both

macromorphological and micromorphological characters fitted with the newly described species *T. osiliensis*, which is now considered to be conspecific with *T. sulfureoides* (Saar & Voitk 2015). After this finding, we examined a dried, previously unidentified *Tricholomopsis* specimen collected from Peloponnese (Mt. Mainalo; *A. cephalonica* forest) in 2001 and preserved in the Mycetotheca ATHUM, whose characteristics led to the same species. This specimen is currently the oldest collection of *T. sulfureoides* from Europe and the first record from Greece. Molecular identification confirmed our determinations. In 2015 a new sample of *T. sulfureoides* was collected in *A. cephalonica* forest of Mt. Parnassos National Park, at a different site from the one of the 2012 collections.

The study of microscopic characters in Greek specimens of *T. sulfureoides* reveals a high fluctuation in spore size and shape. The specimens possess a broader range of spore length in comparison to all other European collections, whereas the spore width is smaller reflecting thus higher Q values. *T. sulfureoides* is a species showing big variability in spore length measurements and consequentially in spore shape, both between different collections and within the same collection, as it was revealed from the Greek specimens, our observations being in accordance with those of Holec (2012b) and Holec & al. (2019). In addition, Holec & al. (2019) notified that the newly described species *T. badinensis* and *T. sulfureoides* have similar and overlapping microscopic characters though the spores of *T. badinensis* typically look slenderer than those of *T. sulfureoides*. All three Greek specimens of *T. sulfureoides* have spores with slightly higher Q values resembling more those of *T. badinensis*. This finding strengthens the conclusion of Holec & al. (2019) that there are no distinguishing microscopic characters between the two species to be used for accurate diagnosis. In Table 3 a comparison of spore sizes from European collections of *T. sulfureoides*, the American type collection of *T. sulfureoides*, and the Slovak species *T. badinensis* is given.

In Greek specimens two types of cheilocystidia are observed: from ovoid to obovoid, broadly fusiform to broadly clavate, but also cylindric or irregularly lageniform, some with usually one projection, up to 15 μm. Holec (2012b) described also clavate cystidia with such a projection, up to 30 μm from the Slovak collection, while Smith (1960) referred fingerlike projections in cheilocystidia of *T. bella* A.H. Sm., a species that he considered to form a species complex together with *T. thompsoniana* (Murrill) A.H. Sm. and *T. sulfureoides*. Pleurocystidia in studied material are frequent, some emerging deep from trama.

TABLE 3. Comparison of spore sizes of *Trichomolopsis sulfureoides* from Europe and USA (holotype) and *T. badinensis*.

VOUCHER	COUNTRY	SPORE MEASUREMENTS	REFERENCE
Tricholomopsis sulfureoides			
JV 26540F	Estonia	6.0–6.9–8.2 × 4.5–5.3–6.0 μm, Q = 1.2–1.33–1.5	Vauras 2009 [as *T. osiliensis*]
PRM 899184	Slovakia	8.0–9.6 × 5.6–6.0 μm, Q = 1.33–1.85 (mean = 1.52)	Holec 2012b [as *T. osiliensis*]
PRM 944836 PRM 945185 PRM 946096 PRM 946095 BRNU DD BIA17/401	Poland	6–8 × 4.5–6 μm	Holec & al. 2019
ATHUM 9526	Greece	6.9–9.6 × 4.1–5.6 μm, Q = 1.41–1.71–2.08	This study
ATHUM 9527	Greece	6.1–9.5 × 3.8–5.2 μm, Q = 1.44–1.72–2.34	This study
ATHUM 9548	Greece	6.1–10.1 × 4.2–5.9 μm, Q = 1.34–1.6–2.09	This study
NYSf 3116.10 [HT]	USA	5.5–8.5 × 3.5–6 μm, Q = 1.2–1.7 (mean = 1.4)	Saar & Voitk 2015
Tricholomopsis badinensis			
PRM 899423 PVKU 1007 PVKU 1439	Slovakia	7 –9 × 4.5–6 μm, Q =1.27–1.80 (mean = 1.46–1.60)	Holec & al. 2019

Fruitbodies of Greek collections of *T. sulfureoides* have yellow tones, almost concolorous pileus and stipe, without any trace of red or violet tinges. In mature basidiomata orange spots can be seen while scales on pileus are absent. Generally, the macromorphology of the specimens fits with the species descriptions, apart from collection ATHUM 9548, where the stipe is covered by scarce brownish contrasting fibrils, a feature which is not mentioned in descriptions of the European specimens. Moreover, it is important to mention that all fruitbodies evolved a dark purplish red coloration in pileus and stipe after drying.

T. sulfureoides grows on temperate and boreal locations of N. America and Europe on fallen trunks of *Picea* and *Abies*, in virgin, old growth forests (Slovak

and Polish collections) or at least in natural forests (Estonian collections; one of the two Estonian localities is a protected area) (Smith 1960; Vauras 2009; Holec 2012b; Holec & al. 2019). In Asia, there is only one specimen recorded, from China (Holec & al. 2019). Concerning Greek specimens, two of the three localities are situated inside a natural reserve in the oldest National Park of Greece in Mt. Parnassos, Central Greece. The third locality, where the earliest European collection of *T. sulfureoides* from Europe was found, is in Mt. Mainalo in Peloponnese, which, although it is included in the European Network Natura 2000 protected areas, it never had a stable status of protection. All Greek specimens were found on dead wood of A. *cephalonica*, the endemic fir of Greece, a fact which strengthens the assessment that *T. sulfureoides* grows in Europe on dead wood of conifers. Importantly, Greek collection sites seem to represent the most southern distribution of the species in Europe (there are no corresponding data from Spain). Our findings indicate that it is important to search for *T. sulfureoides* all over Europe and also worldwide, in order to understand its distribution and its ecological preferences, and thus give a satisfactory answer to the question posed by Vauras & al. (2012), why this species has not been recorded earlier in Europe.

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MYCOTAXON

Ganoderma gibbosum newly recorded from Pakistan

Aisha Umar¹*, Shakil Ahmed¹, Eman Alhomaidi²

¹ Institute of Botany, University of the Punjab Quaid-e-Azam Campus, 54590, Lahore, Pakistan
² Department of Biology, College of Science, Princes Nourah bint Abdulrahman University
   P.O. Box 84428, Riyadh 11671, Saudi Arabia

* Correspondence to: ash.dr88@gmail.com

Abstract—*Ganoderma gibbosum* is a new record for Pakistan, identified by morphological and molecular data. DNA sequence analysis of ribosomal 5.8S rRNA gene including the flanking nuclear ribosomal DNA-ITS regions confirmed the Pakistani specimens as *G. gibbosum*. Morphologically, the specimens are characterized by their non-laccate sessile basidiomata, truncate, short fine minute inter walled pillars, elongated basidiospores (7.5–10.2 × 4.6–5.9 μm), and greyish to greyish-brown dull pileal surface.

Key words—*Elfvingia, Ganodermataceae,* morphology, polypore, taxonomy

Introduction

Imazeki (1952) divided *Ganoderma* into two subgenera: *G.* subg. *Ganoderma* [typified by *G. lucidum* (Curtis) P. Karst.] and *G.* subg. *Elfvingia* (P. Karst.) Imazeki [typified by *G. applanatum* (Pers.) Pat.] (Gottlieb & Wright 1999a,b). Species of *G.* subg. *Ganoderma* are laccate, with palisade and inflated hyphal ends (Zhao & Zhang 2000), whereas, species of *G.* subg. *Elfvingia* are non-laccate, with palisade absent and a dull basidiome (Smith & Sivasithamparam 2000; Richter & al. 2015).

A few species reported from Pakistan in *G.* subg. *Elfvingia* are *G. australe* (Fr.) Pat., *G. tornatum* (Pers.) Bres., and *G. lipsiense* (Batsch) G.F. Atk. (Li & al. 2010). Taxonomic studies regarding *G.* subg. *Elfvingia* rely on macro- and micromorphological characters to identify and delineate the species. However,

morphological concepts are insufficient to correctly define *Ganoderma* specimens (Moncalvo & Ryvarden 1997), as influenced by environmental conditions and geographic origin (Torres-Torres & Guzmán-Dávalos 2012). The use of the "biological" and "phylogenetic" species recognition concept has significantly contributed in genus delimitation and species identification (Moncalvo & al. 1995; Zhou & al. 2015).

Such studies are still restricted due to limited numbers of specimens, geographic regions, validated material, and misidentifications; and the public database records constitute major complications that are obstacles preventing the drawing of robust phylogenetic conclusions (Fryssouli & al. 2020). Evolutionary relationships among phylogenetically related taxa from different continents are quite imperfectly known, with very important gaps revealed in geographical distributions as well (Welti & Courtecuisse 2010). ITS data might be helpful in resolving the taxonomy of the *G. gibossum* complex, and comprehensive studies are necessary for any taxonomic decision (Moncalvo 2000).

This study aims to prove that the specimens from Pakistan used in this study were correctly determined as *Ganoderma gibbosum*, through detailed observations of both morphological and molecular characters.

Materials & methods

Studied materials

Specimens were collected in the 2018–19 monsoon season from Changa Manga Forest, District Kasur, Punjab, and from University of the Punjab, New Campus, Lahore, Pakistan. Both sites are covered by *Dalbergia sissoo* Roxb. ex DC. and *Acacia nilotica* (L.) Willd. ex Delile. The average annual rainfall is 1232 mm and the temperature 24°C (Ahmad & al. 2014). Size, shape, and color of basidiomata were noted. The color descriptions followed Munsell (1975). Microscopic structures were observed from cross sections of the basidiome after soaking in KOH (5% w/v), stained with Congo red (1%) and viewed under a MX4300H compound light microscope (Meiji Techo Co., Ltd., Japan). The data of micromorphological features were recorded from at least 30 measurements of face view and side view at a magnification of 100X. Basidiospore measurements are presented as length × width (Nagy & al. 2010). Morphological descriptions of the microscopic features use (in part) the terminology defined by Torres-Torres & Guzmán-Dávalos (2012). The specimens were deposited in Mycology Herbarium, Laboratory of Mycology, Institute of Botany, Academy of Sciences of the Republic of Uzbekistan, Tashkent 100125, Uzbekistan (TASM-ZSH).

DNA extraction, amplification, and sequencing

Modified CTAB procedure was followed to extract total genomic DNA from the dried specimens (Doyle & Doyle 1987). ITS1+5.8S+ITS2 rDNA region (henceforth referred to as the ITS region) was used to study the target specimens. This region was amplified by using primers ITS1 and ITS2 (White & al. 1990). Reaction mixtures (20 µl) contained 0.5 µl template DNA, 8.5 ml distilled water, 0.5 µl of each primer, and 10 ml PCR mix [DreamTaqGreen PCR Master Mix (2 X), Fermentas]. Amplification conditions were 35 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Amplified PCR products were purified and sequenced by TSINGKE Co. Ltd. (China).

Molecular phylogeny

The consensus sequence was generated from both ITS1 and ITS2 in BioEdit version 7.2.5 (Hall 1999) in the lab of Princes Nourah bint Abdulrahman University (Department of Biology, College of Science) and then searches were performed at the National Center for Biotechnology Information (NCBI) web site using BLASTn. The sequences generated during this study were submitted and deposited in GenBank (OM350446, OM350473). A dataset of 41 ITS based accessions were taken from published literature and downloaded from GenBank. Sequences were aligned and edited by using ClustalX 2.1 (Larkin & al. 2007) and BioEdit (Hall 1999). GenBank and newly generated sequences were aligned with MAFFT v. 10 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh & Standley 2013). The alignment was manually edited and trimmed at 590 positions. These sequences representing 19 taxa were used to construct the phylogenetic tree. *Tomophagus colossus* was selected as outgroup (Zhou & al. 2015). MEGA 10.0 was used to run the maximum likelihood phylogenetic analysis with 1000 bootstrap replicates (Tamura & al. 2011).

Results

Molecular phylogeny

The phylogenetic tree produced by maximum likelihood analysis of cladistically informative sites is depicted in Fig. 1. The Pakistani *G. gibbosum* sequences (U2, U21) from our study made a well-supported clade with South Korean *G. gibbosum* together with *G. ellipsoideum* and *G. eickeri*; but less close to Colombian *G. gibbosum* (Fig. 1).
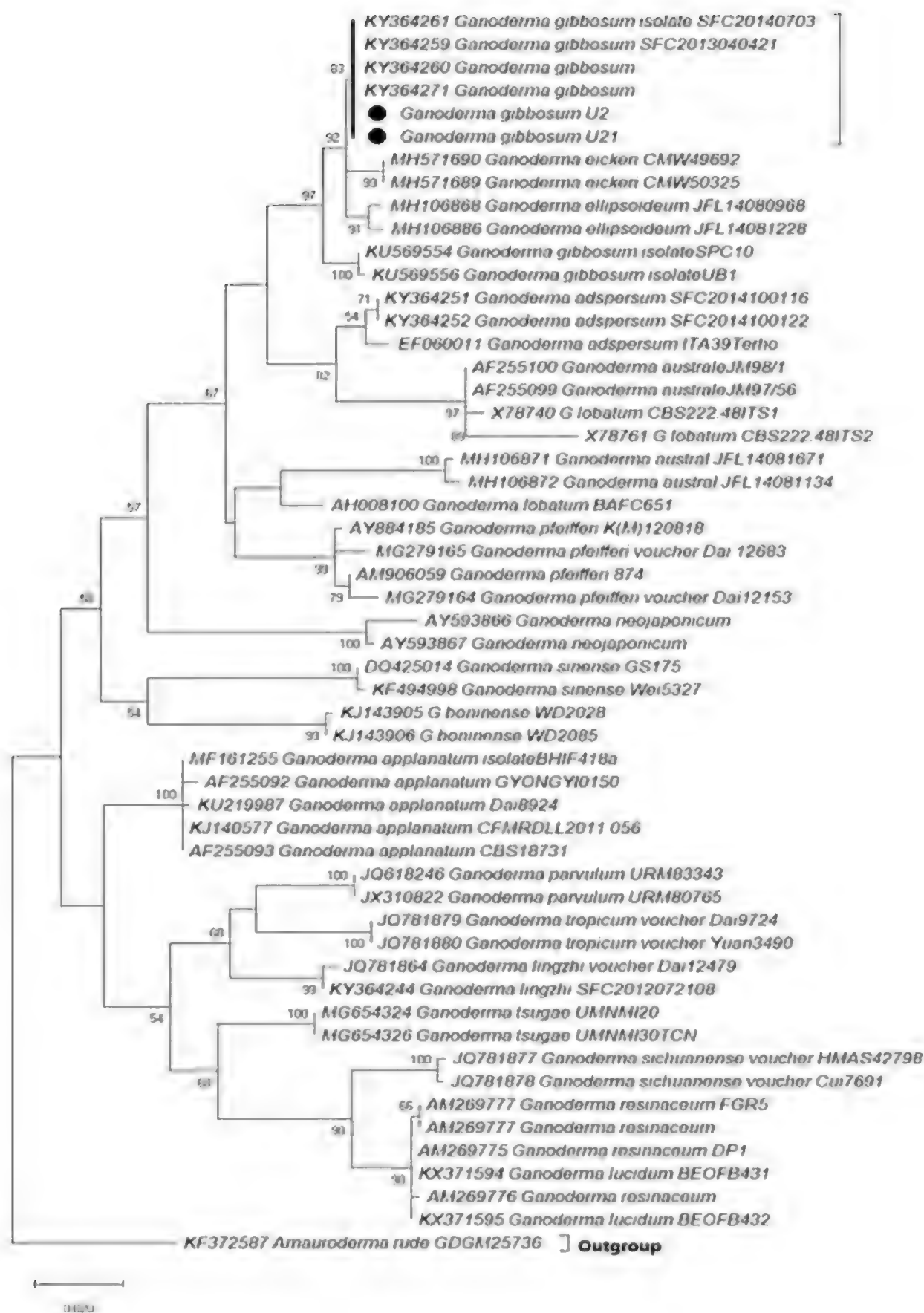
FIG. 1. Phylogenetic tree of *Ganoderma* based on ITS rDNA sequences generated by maximum likelihood, with *Amauroderma rude* as outgroup. Bootstrap values >50% are shown at the branches. Newly obtained sequences are marked with •.

Taxonomy

Ganoderma gibbosum (Blume & T. Nees) Pat., Ann. Jard. Bot. Buitenzorg,
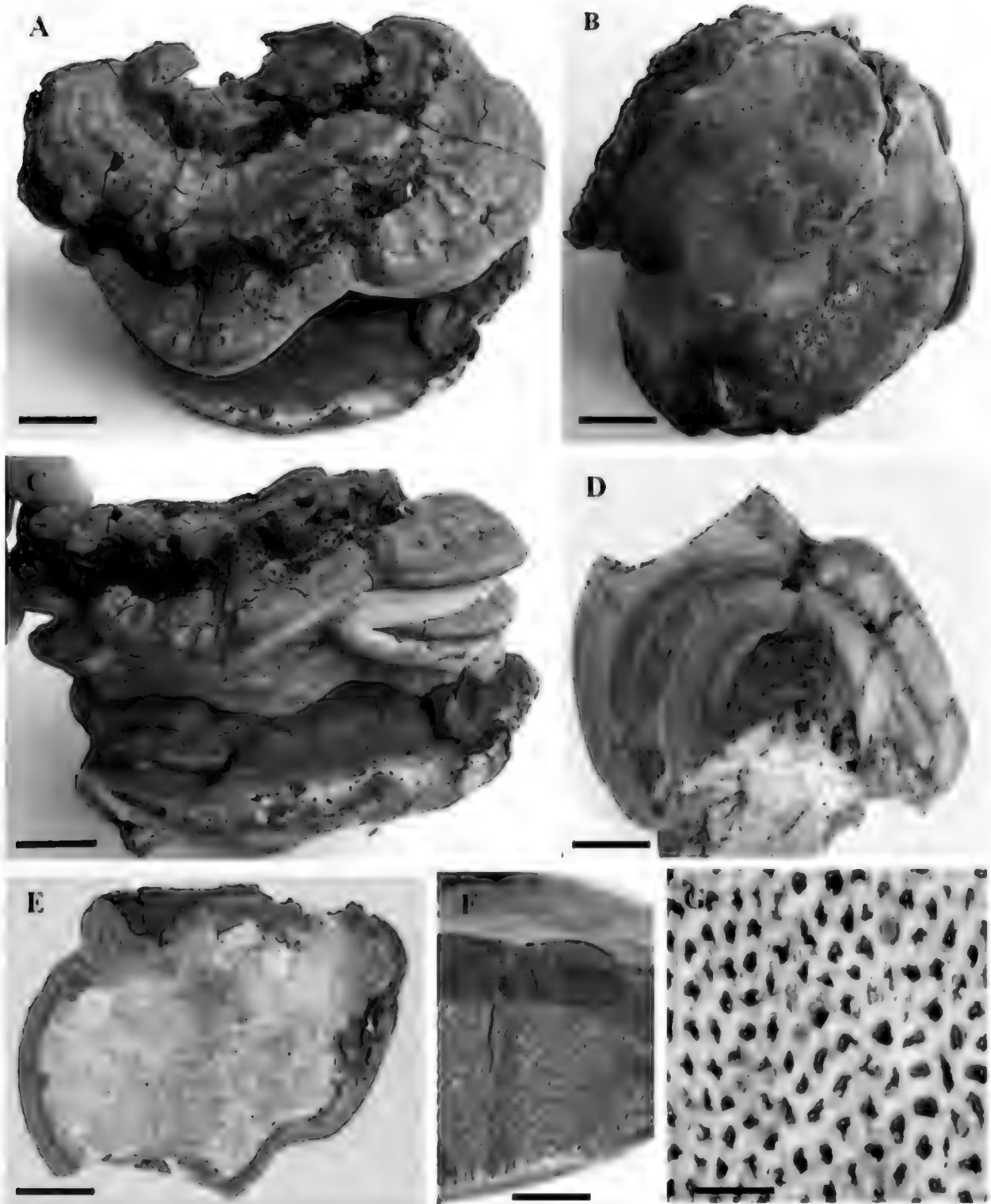suppl. 1: 114 (1897)   FIGS 2, 3

FIG. 2. *Ganoderma gibbosum* (TASM-ZSH091). A–C. Basidiomata; D, E. Pore surfaces and pores; F. Section of context and tubes. Scale bars: A–C = 2 cm; D, E = 2 mm; F = 1 mm.
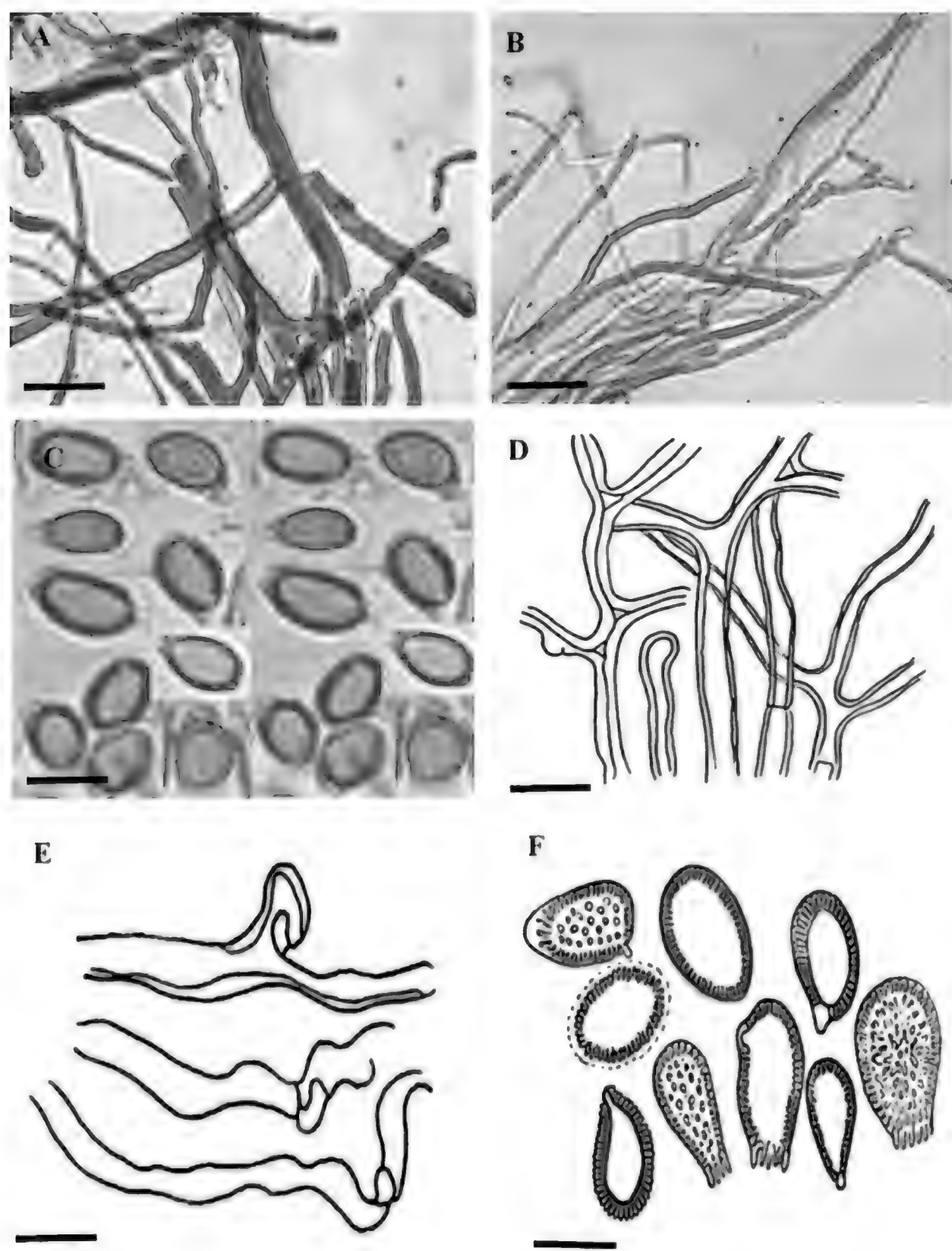
Fɪɢ. 3. *Ganoderma gibbosum* (TASM-ZSH091). A, B. Skeletal hyphae; C, D. Generative hyphae; E, F. Basidiospores. Scale bars: A–D = 5 μm; E, F = 10 μm.

BASIDIOMATA sessile, annual, woody, non laccate; PILEUS 9.0–9.4 × 4.5–4.8 cm, 4.5–4.8 cm thick, hard, sub orbicular, multilayered thick, whitish light brown to grayish brown, crust, concentric zones, turberculate bumps on surface, rivulose depressions, ridges; MARGIN several undulations, irregularities, 3 to 7 mm thick, concolorous to pileus; PORES 4–5/mm, light brown, sub circular to circular; TUBES 7–8 mm long, chocolate brown, grayish brown to golden; PILEIPELLIS/hymeniderm light brown to brown, branched cells, apically acanthus; HYPHAL SYSTEM dimitic: 1) skeletal hyphae 4.8–5.2 μm (width), brown, thick-walled, 2) generative hyphae 1.6–2.7 μm (width), hyaline, thin-walled; BASIDIOSPORES 7.5–10.2 × 4.6–5.9 μm, elongate to ellipsoid, light brown to brown, bitunicate eusporium, fine distinct short inter walled pillars, hyaline myxosporium.

SPECIMENS EXAMINED—**PAKISTAN, PUNJAB, Kasur district**, Changa Manga Forest, 31.0500°N 73.4072°E, 200 m a.s.l., gregarious on decayed hardwood, *Dalbergia sissoo* and *Acacia nilotica*, 15 July 2018, Aisha Umar, U21 (TASM-ZSH090; GenBank OM350473). **Lahore district**, Lahore, New Campus, University of the Punjab, 31.4981°N 73.3044°E, 217 m a.s.l., gregarious on hardwood, on living tree trunk of *A. nilotica*, 10 April 2019, Aisha Umar, U2 (TASM-ZSH091; GenBank OM350446).

Discussion

Ganoderma gibbosum is a non-laccate species reported from China, Korea, Vietnam, Laos, Thailand, Japan, Taiwan, India, Indonesia, Australia, USA, Mexico, Puerto Rico, Costa Rica, Colombia, Ecuador, Peru, Argentina, Brazil, and French Guyana (Blume & Nees von Esenbeck 1826; Saccardo 1888; Parmasto 1986; Moncalvo 2000; Cong 2010; Dai & al. 2011; Rojas & al. 2018; Hapuarachchi & al. 2018, 2019; Fryssouli & al. 2020). Dubious status and relationships in Neotropical *G. gibbosum* complexes is due to limited sequence data. Genetic approaches (Cabarroi-Hernández & al. 2019; Tchoumi & al. 2018) provide valuable information to resolve phylogenetic patterns and mitigate limitations of orthodox methodologies (Fryssouli & al. 2020). Nevertheless, one of the possible hypotheses for parasitic and saprotrophic fungi on woody substrates, like *Ganoderma*, is that they may travel to long distances on introduced plants (Moncalvo 2000).

Our collections of *Ganoderma* from Pakistan agree with the description provided by Ryvarden (2000), and we report *G. gibbosum* as a new record based on molecular and morphological evidence. *Ganoderma gibbosum* from Pakistan was closely related to *G. gibbosum* and *G. eickeri* in the phylogeny inferred from the concatenated sequence data set. These species in *G.* subg. *Elfvingia* are easily distinguished by some macro- and microscopic characters of their basidiomes

(Tchoumi & al. 2018). Non-laccate species are heavily agglutinated and the massive matrix of pileal crust often obstructs the observation of discriminating features (Fryssouli & al. 2020).

Basidiomata of *Ganoderma gibbosum, G. ellipsoideum, G. adspersum, G. australe*, and *G. lobatum* [but not *G. eickeri*] are annual (Tchoumi & al. 2018; Hapuarachchi & al. 2019), sessile, non-laccate, woody, reniform, applanate to flabellate to dimidiate in shape, similar to our Pakistani specimens of our species. Basidiomata of our specimens are dark greyish to grey, while other non-laccate species range from reddish to brown in color. The pileus zones of *Ganoderma gibbosum, G. ellipsoideum, G. adspersum,* and *G. australe* are concentrically sulcate with tuberculate bumps and rivulose depressions, ruptured crust, margin concolourous with the pileus (Tchoumi & al. 2018; Hapuarachchi & al. 2019), like our Pakistani specimens.

The context of *G. eickeri* and *G. lobatum* (Tchoumi & al. 2018; Hapuarachchi & al. 2019) is soft, lightly woody, and homogenous chocolate brown, compared with dry, woody triplex in *G. ellipsoideum* and duplex in *G. gibbosum, G. adspersum,* and *G. australe* (Hapuarachchi & al. 2019), like our Pakistani specimens. Thickness of context is 4 mm to 12 mm in *G. lobatum* (Gottlieb & Wright 1999a), 3 cm in *G. eickeri* (Tchoumi & al. 2018), 1.5 cm in *G. gibbosum*, 2.5 cm in *G. ellipsoideum*, and 1.5 cm in *G. australe* (Hapuarachchi & al. 2019), whereas 6–9 mm in our Pakistani specimens. Basidiospore size of *G. adspersum* is 8.5–9.6–10.5 × 5.4–6.1 μm (Hapuarachchi & al. 2019), *G. australe* 7.6–9.2–10.8 × 5.3–7.6–7.9 μm (Bi & al. 1993; Hapuarachchi & al. 2019), *G. ellipsoideum* 6.1–7.3 × 3.7–4.6 μm, *G. eickeri* 8.5-11 × 5-7 μm (Tchoumi & al. 2018), *G. lobatum* 7.5-11 × 5-7 μm (Steyaert 1980), and *G. gibbosum* 6.9–7.6 × 4.6–5.6 μm (Hapuarachchi & al. 2019), all similar to our specimens (7.5–10.2 × 4.6–5.9 μm). These are ranged from ovoid to ellipsoid, bearing fine, short and distinct inter walled pillars with overlaid hyaline myxosporium. Basidiospores disseminate by wind and human activities (Gonthier & al. 2004; Moncalvo & Buchanan 2008), which may facilitate the broad geographic distribution of *Ganoderma* species, especially those present in more than one locality (Gonthier & al. 2004; Moncalvo & Buchanan 2008; Tchoumi & al. 2018).

On angiosperms, *Ganoderma eickeri* originates from South Africa (Tchoumi & al. 2018). However, the ITS data of *G. eickeri* are closely related to *G. gibbosum.* Sequences of *G. ellipsoideum* subclade were mainly labelled as "*G. gibbosum*", "*G. australe*", and "*G. applanatum*", and originated from broad geographic distribution (south and east Asia, Oceania) on both eudicots and monocots, but not on gymonsperms (Fryssouli & al. 2020).

We conclude that morphological plasticity and substantial overlapping of "diagnostic" characters are prevalent in these 'dull' taxa, so that their taxonomic importance is dubious. This suggested that laccate or non laccate traits can be used for demarcation of *Ganoderma* species at the subgeneric level, but do not always reveal the true phylogenetic relationships.

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MYCOTAXON

Molecular studies of *Flavopunctelia* and *Punctelia* species and their *Trebouxia* photobiont from the Himalayas, India

BHARATI SHARMA[1], SHAHNOOR FATIMA[1], KUNHIRAMAN C. RAJESHKUMAR[1*],
PAVEL ŠKALOUD[2], PRADEEP KUMAR DIVAKAR[3], SUBHASH GAIKWAD[1],
PARAYELIL ABDULSALIM ANSIL[1], ARSHA S. MOHAN[4], STEPHEN SEQUEIRA[4]

[1] *Biodiversity & Palaeobiology (Fungi & Lichens) Group, MACS Agharkar Research Institute, Pune, 411004, Maharashtra, India*

[2] *Algal Speciation & Evolution Lab, Department of Botany, Charles University in Prague, Benatska 2, CZ-12801, Praha 2, Czech Republic*

[3] *Departamento-de Farmacología, Farmacognosia y Botánica, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain*

[4] *Department of Botany, Maharaja's College, Ernakulam 682011, Kerala, India*

* CORRESPONDENCE TO: *rajeshfungi@gmail.com*

ABSTRACT— *Flavopunctelia* and *Punctelia* species collected from Pir Panjal Ranges of Himachal Pradesh, India were studied by morphological, chemical, and phylogenetic methods. Analyses based on a concatenated ITS and LSU data set, confirmed the position of the three Indian taxa within the *Parmotrema*-clade in *Parmeliaceae*. The morphology-based taxonomic study reiterated the presence of cryptic species in *Flavopunctelia* with highly similar morphological characters in different species. The type species, *F. flaventior,* formed a well-supported monophyletic clade adjacent to *F. soredica*. In *Punctelia*, *P. borreri* was also established as a unique lineage along with *P. perreticulata*. RPB1 data were not interpreted due to data deficiency. The ITS sequence data and analyses showed *Trebouxia* photobionts in all the parmelioid species; that of *P. borreri* was identified as *T. gelatinosa*, but the photobionts of *F. flaventior* formed an exclusive clade of an apparently undescribed species of *Trebouxia*, and the photobiont of *F. soredica* formed an unresolved basal clade to the *Trebouxia* sp. of *F. flaventior*.

KEY WORDS—*Lecanorales*, lichens, symbionts

Introduction

Parmeliaceae is one of the most prominent families of lichen-forming fungi represented by 69 genera and about 2726 species (Divakar & al. 2017). Indian *Parmeliaceae* include 43 genera and about 368 species as currently circumscribed (Singh & Sinha 2010, Sinha & al. 2018). A lichenicolous life-style originated independently three times from lichenized ancestors within the family about 24 Mya (Divakar & al. 2015). Crespo & al. (2010) redefined the generic boundaries of parmelioid lichens (*Parmeliaceae, Lecanorales*) based on molecular, morphological and chemical evidence, recognizing 27 genera within eight major clades. That study established a stable framework for the generic classification within the family. The *Parmotrema*-clade is a major clade in *Parmeliaceae*, and includes *Austroparmelina, Canoparmelia, Flavoparmelia, Flavopunctelia, Nesolechia, Parmotrema,* and *Punctelia.* Members of that clade are distinguished by a predominant diagnostic cell wall polysaccharide, isolichenan. Most have a pored epicortex, some have pseudocyphellae, and they contain either atranorin or usnic acid as a cortical pigment. In the last decade, several studies on *Parmeliaceae* have been conducted from across the world have expanded or emended the generic and species boundaries based on morphological and molecular sequencing and phylogenetic analyses (Divakar & Upreti 2003, 2005, Thell & al. 2005, Crespo & al. 2010, Divakar & al. 2010, Lendemer & Hodkinson 2010, 2012, Alors & al. 2016).

During 2018 in the monsoon seasons, several surveys were conducted into relatively pristine habitats of the Western Himalayas to explore the diversity of Indian *Parmeliaceae.* The collected specimens were subjected to morphological studies, molecular sequencing, and phylogenetic analyses that established the identification of different species within *Flavopunctelia* and *Punctelia.* This study attempts to unravel the diversity of Indian parmelioid lichens through modern taxonomic approaches that include molecular, morphological, and chemical data using material from the diverse habitats of the Indian Himalayan region.

Materials & methods

Sample collection

Field surveys were conducted in the Pir Panjal Ranges near Hamta Pass (32.2706°N 77.3481°E) in Himachal Pradesh, India, during the 2018 monsoon season. We collected 40–50 fresh specimens from five different microhabitats. Minimal sampling protocols were followed to conserve the in situ diversity. The samples were dried under shade and stored in brown paper covers and transported to the laboratory for morphological and molecular study. Fresh thalli were stored in 4°C specifically for molecular studies

in order to avoid cross contamination from fast growing saprophytic fungi. After preliminary morphological studies, 30 specimens were selected and subjected to molecular studies. Prior to DNA isolation, manual cleaning was done using a brush to remove plant parts and bryophytic remnants and further washed in distilled water so as to facilitate the recovery of high-quality DNA. Specimens were conserved in the Ajrekar Mycological Herbarium, Agharkar Research Institute, Pune, India (AMH).

Morphology & chemical analyses

Thallus morphology of all the samples were studied using a Nikon binocular stereomicroscope (Model SMZ-1500 with Digi-CAM, Japan). Thallus and lobes were measured, thallus colour, lobe shape, soralia, isidia, cilia, rhizhines, and other features of the upper and lower cortices and thalli such as apothecia, pseudocyphellae, and pycnidia were observed and noted. Thallus sections were made using a razor blade and mounted in lactic acid cotton blue (with gentle heating over the flame) for microscopy. Morphological characteristics were elaborated and compared with standard taxonomic references (Divakar & Upreti 2005, Crespo & al. 2010). Chemical profiles were studied by thin layer chromatography (TLC) following standard protocols (Culberson 1972, White & James 1985, Orange & al. 2001).

DNA isolation, polymerase chain reaction and sequencing

Total genomic DNA from the lichen thalli was isolated by a modified CTAB method (Cubero & al. 1999, Porebski & al. 1997). Additionally, the sorbitol wash method (Inglis & al. 2018) was also used for achieving quality DNA from samples having dark pigmentation. A DNA isolation kit (FavoPrep™ Plant Genomic DNA Extraction Mini Kit, Taiwan) protocol was also used as an alternative for challenging lichen samples. Quantification of DNA was made using NanoDrop ND-1000 spectrophotometer V3.8.1 (Thermo scientific, USA) and quality was ensured for further PCR studies. For amplifying internal transcribed spacer regions (ITS) from the photobiont, the primer pair ITS1T and ITS4T (Kroken & Taylor 2000) was used. For amplifying ITS of the mycobiont, the primer pair ITS5 and ITS4 (White & al. 1990) and also ITS1F (Gardes & Bruns 1993) were used. The partial 28S nrDNA (LSU) was amplified using the primer pair LROR and LR5 (Vilgalys & Hester 1990). The protein coding RPB1 gene was amplified using the primers gRPB1-A (Stiller & Hall 1997) and fRPB1-C (Matheny & al. 2002). The PCR reactions (25 µl) contained 10× buffer (containing 100 mM Trizma/HCL, pH 8.3 at 25°C, 500 mM KCL, 15 mM MgCl2, 0.01% (w/v) gelatin), 0.2 mM each dNTP, 0.5 µM each primer, 1unit Taq DNA polymerase (Sigma–Aldrich) and 1–10 ng genomic DNA extract. The amplifications for ITS, LSU rDNA and RPB1 were carried out in an automatic thermocycler ProFlexTM PCR system (Applied Biosystems, USA). Thermal cycling parameters used for amplification were: initial denaturation at 95°C for 5 min; then 30 cycles of: {[i] 94°C for 1 min; [ii] either 45–50°C (ITS5–ITS4) for 30 s, or 54–56°C (ITS1F–ITS4) for 1 min, or 54–56°C (LSU rDNA) for 1 min, or 56°C (RPB1 nrDNA) for 50 s, or 54–56°C (Photobiont ITS); [iv] 72°C for 90 s}; with a final extension at 72°C for 10 min. The PCR products were purified with StrataPrep PCR Purification Kit (Agilent Technologies, TX, USA) and

sequenced with the same primers using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequencing reactions were run on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analyses

Sequences with high similarity percentages were determined from a BLASTn search to find the closest matches with taxa and recently published data in GenBank. Sequences generated from different primers of the three genes (ITS, LSU, RPB1) were analysed with other sequences (following Crespo & al. 2010) retrieved from GenBank. The multiple sequence datasets were aligned with MAFFT v.7 at the web server (http://mafft.cbrc.jp/alignment/server; Katoh & al. 2019), and manually edited where necessary in BioEdit v.7.0.9.0 (Hall 1999). The phylogeny website tool "ALTER" (Glez-Peña & al. 2010) was used to transfer the alignment file in to PHYLIP format for RAxML analysis. Phylogenetic analyses of both individual and combined aligned data were performed with the maximum likelihood (ML) method with support in nodes calculated with bootstrap analyses (BS) and Bayesian analysis (PP). Phylogeny was inferred using the program RAxML v8.1.11 (Stamatakis 2006; Stamatakis & al. 2008). Based on the J-Model test, the best fit model of nucleotide substitution 'GTRGAMMA+I' was implemented, with locus-specific model partitions treating all loci as separate partitions, and evaluated nodal support using 1000 bootstrap pseudo-replicates. Exploratory analyses using alternative partitioning schemes resulted in identical topologies and highly similar bootstrap support values. For the Bayesian tree sampling, the concatenated ITS and LSU data set was partitioned as described in the ML analysis in siMBa (Mishra & Thines 2014), specifying the best fitting model, and allowing unlinked parameter estimation and independent rate variation. Posterior probabilities (PP) were estimated by sampling trees using a variant of the Markov Chain Monte Carlo (MCMC) method. Phylogenetic trees were sampled every 1000th generation (resulting in 4000 total trees) in 4,000,000 generations from running of six simultaneous Markov chains. The first 1000 trees, which contained the burn-in phase of the analyses were discarded. The remaining 3000 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. Based on the likelihood profile, the first 25% trees were discarded as burn in. Only clades with BS ≥50% and PP ≥0.95 were considered as supported. Phylogenetic trees were visualized using the program FigTree 1.4.0. (Rambaut 2014). Trees were edited using Microsoft Power Point. DNA sequences that were newly generated in this study were deposited in GenBank. For the phycobiont phylogeny, sequences were retrieved based on the studies by Kroken & Taylor (2000) and Škaloud & al. (2018).

Results

Phylogenetic analyses

Based on a megablast search of GenBank nucleotide database, eight of our *Flavopunctelia flaventior* AMH 18.16(5), AMH 18.383(15), AMH 18.26(5),

AMH 18.17(5), AMH 18.18(5), AMH 18.15(5), AMH 18.293(7), AMH 18.385(15) had highest ITS similarity (99%, no gaps) to three *F. flaventior* isolates from California (isolates 19, MN006786; 18, MN006785; 17, MN006784); and highest LSU similarity with *F. flaventior* from Spain (AY578923; 99%, no gaps), *F. flaventior* (JN939611.1; 99%, no gaps) and *F. soredica* (JN939613; 99%,1 gap).

Similarly, our other *Flavopunctelia* specimen. AMH 18.66(7) had highest ITS similarity to *Flavopunctelia soredica* (AB623069; 100%, no gaps), *F. soredica* (AY773128; 99%, no gaps); and highest LSU similarity with *F. soredica* (GU994600; 99%, no gaps), *F. flaventior* (AY578923; 99%, no gaps), and *F. flaventior* (JN939613; 99%, 1 gap).

Our *Punctelia* specimens AMH 18.300(7), AMH 18.141(14), AMH 18.137(10) had highest ITS similarity with *P. borreri* (DQ394373; 100%, no gaps), *P. borreri* (GU593038; 99%, no gaps), and *P. borreri* (MG231804; 100%, no gaps); and highest LSU similarity (99%, no gaps) with *P. borreri* (AY578954), *P. subrudecta* (JN939641), and *P. subrudecta* (AY578955).

The combined sequence data of the parmeliod species examined was analyzed with the taxa in *Parmotrema*-clade to determine the species placement (Fig. 1). The tree was rooted with *Xanthoparmelia conspersa*. The analysed dataset comprised LSU (956 bp) and ITS (521 bp) sequence data (a total of 1480 characters including gaps) for 56 taxa. The best RAxML tree with a final likelihood value of −9082.272963 is presented. The matrix had 576 distinct alignment patterns, with 18.57% undetermined characters or gaps. Estimated base frequencies were: A = 0.237735, C = 0.237091, G = 0.286275, T = 0.238899; substitution rates AC = 1.477097, AG = 2.539196, AT = 2.151437, CG = 0.603377, CT = 6.827132, GT = 1.000000; gamma distribution shape parameter α = 0.475632. Phylogenetic trees were sampled every 1000th generation (resulting in 4000 total trees) in 4,000,000 generations from the running of six simultaneous Markov chains. The first 1000 trees, which contained the burn-in phase of the analyses were discarded. The remaining 3000 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. Maximum likelihood and Bayesian analyses resulted in similar topologies. The phylogeny showed that *Flavopunctelia* species in this study aligned with *F. flaventior* collected from California USA (Samples 17(MN006784), 18(MN006785), 19(MN006786) formed a well-supported monophyletic clade adjacent to *F. soredica* (BS=99%, PP=1). The species of *Punctelia* aligned with the *P. borreri* (Hur 030736, GenBank DQ394373) formed a well-supported clade (BS=87%, PP=0.96) (Fig. 1).
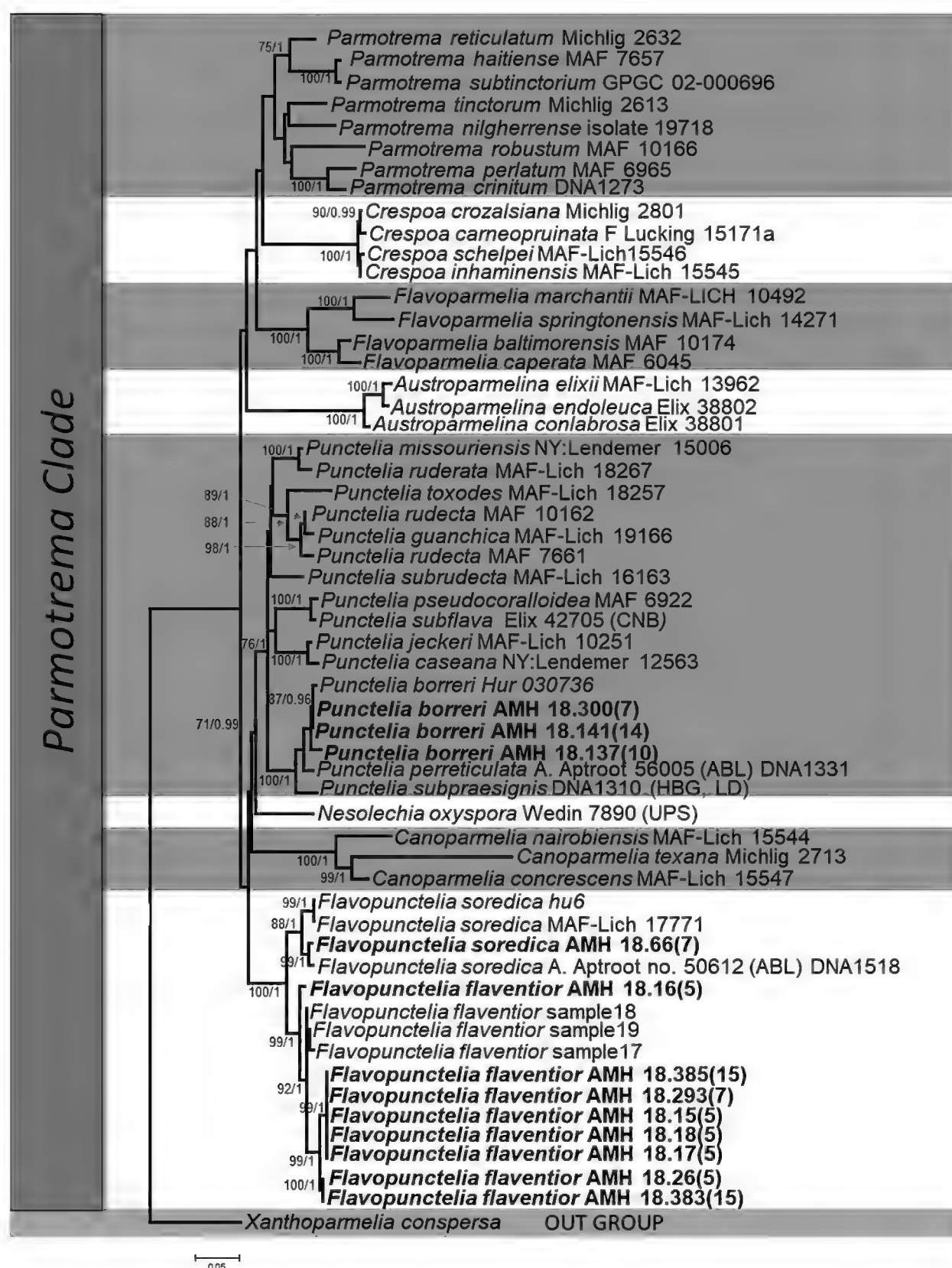
FIG. 1. Phylogram generated from RAxML analyses based on analyses of combined ITS and LSU sequence data for the *Parmotrema*-clade (*Parmeliaceae*). Bootstrap support values are given for BS ≥50% and PP ≥0.95. The tree is rooted to *Xanthoparmelia conspersa*. The new sequences generated are shown in black and bold.

The evolutionary relationships were estimated from a concatenated, two-locus (ITS, LSU) data matrix for the *Flavopunctelia* and *Punctelia* species (Fig. 1). Among the seven species of *Flavopunctelia* reported worldwide (Index Fungorum 2021), three species have molecular sequence data (Crespo & al. 2010, Thell & al. 2005). Based on the phylograms generated in this study, *Flavopunctelia* species form a distinct lineage within the *Parmotrema*-clade (*Parmeliaceae*) adjacent to *Punctelia*, *Canoparmelia* and *Nesolechia* species with high bootstrap value (BS=100%). Similar to the *Flavopunctelia* clade, *Punctelia* species also formed a monophyletic clade (BS=77%) allied to *Canoparmelia* and *Nesolechia* clades.

Based on a megablast search of GenBank nucleotide database, our *Trebouxia* photobiont from *Flavopunctelia flaventior* AMH 18.16(5), AMH 18.383(15), AMH 18.26(5), AMH 18.17(5), AMH 18.18(5), AMH 18.15(5), AMH 18.293(7), AMH 18.385(15) had highest ITS similarity with *Trebouxia* isolate L1199 (KJ754205; 97%, 1 gap), *T. impressa* isolate IB345 (KY559117; 95%, 5 gaps), *Trebouxia* isolate TAE1 (FJ792798; identities= 360/375 (96%), 0% (2 gaps), *Trebouxia* sp. isolate TAE1 (AF242471; 99%, 1 gap). Our *Trebouxia* from *F. soredica* AMH 18.66(7) had highest ITS similarity with *Trebouxia* isolate TAE1 (FJ792798; 96%, 2 gaps) and *T. impressa* isolate L2239p (KX181276; 96%, 2 gaps). The *Trebouxia* from *Punctelia borreri* AMH 18.137(10), AMH 18.300(7) using the ITS sequence, had highest similarity with *T. gelatinosa* isolate L1780 (KT768205; 99%, 1 gap), *Trebouxia* photobiont (AM159214; 99%, 1 gap), *Trebouxia* isolate TTC1 (FJ792802; 99%, 1 gap), and *T. anticipata* isolate NIES1271 (MK328538; 99%, 0 gap),

The *Trebouxia* photobiont tree is rooted with *Trebouxia* sp. OTU A19 ID 3742. The analysed dataset comprised ITS sequence data of 977 bp characters including gaps for 47 taxa. The best RAxML tree with a final likelihood value of –5247.435430 is presented (Fig. 2). The matrix had 405 distinct alignment patterns, with 36.34% undetermined characters or gaps. Estimated base frequencies were: A = 0.221945, C = 0.237199, G = 0.272429, T = 0.268427; substitution rates AC = 0.979471, AG = 2.791362, AT = 1.783801, CG = 0.518499, CT = 3.658680, GT = 1.000000; gamma distribution shape parameter α = 0.355443. Maximum likelihood and Bayesian analyses resulted in similar topologies.

In our phylogenetic analyses, all the *Trebouxia* species associated with *Flavopunctelia flaventior* were delineated as a major clade along with an undescribed Clade I-08 allied to *Trebouxia* Clade I. Similarly, *Trebouxia* species from *F. soredica* also formed a basal lineage to Clade I-08 along with *Trebouxia* species associated with *F. flaventior*. *Trebouxia* photobionts of *Punctelia borreri* were phylogenetically allied to a well-supported clade of *Trebouxia gelatinosa*.
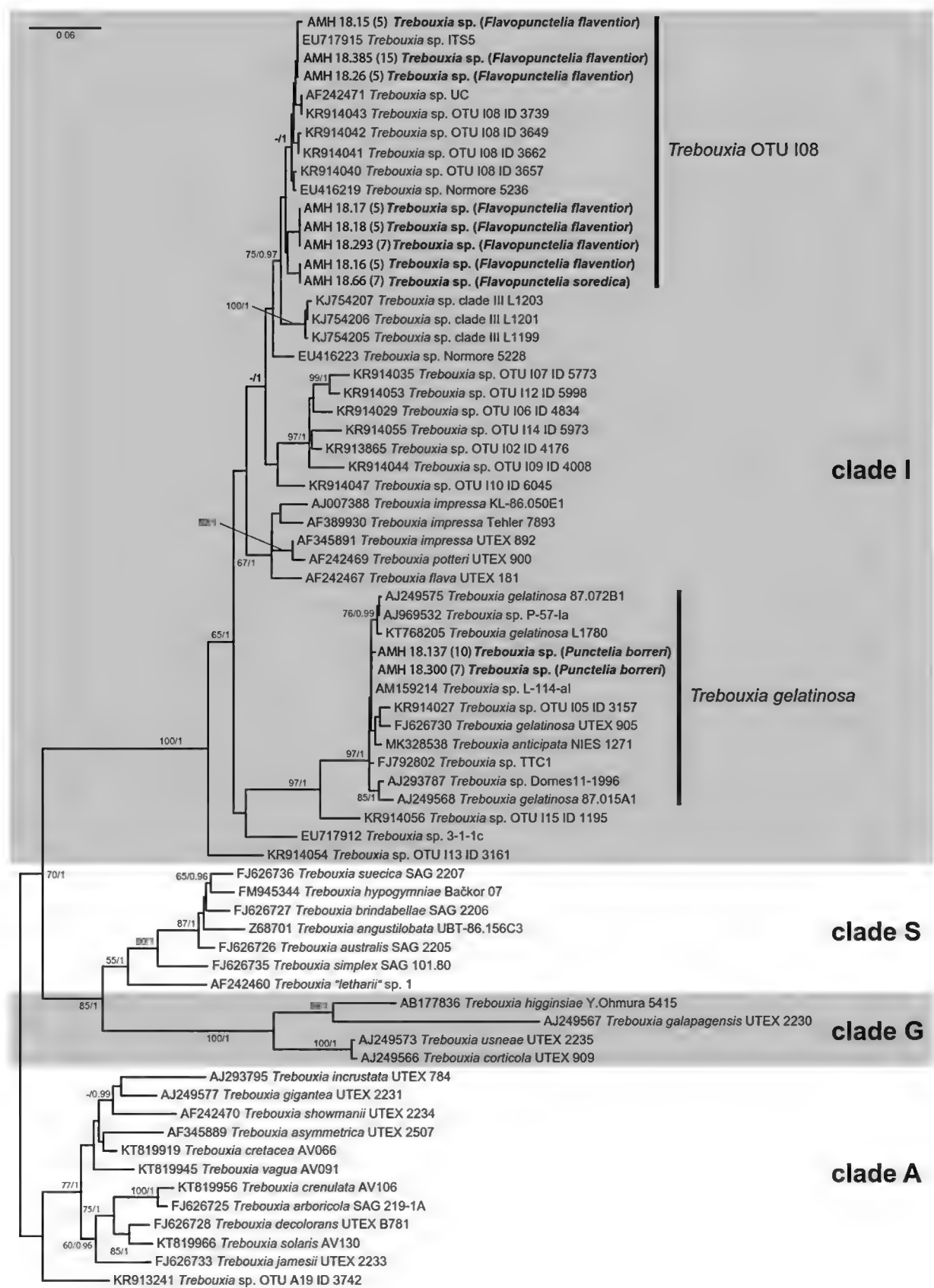
Fig. 2. Phylogram generated from RAxML analyses based on analyses of ITS sequence data for the photobiont *Trebouxia* (*Trebouxiaceae*). Bootstrap support values are given for BS ≥60% and PP ≥0.95. The tree is rooted to *Trebouxia* sp. (Clade A). The new sequences generated are shown in blue and bold.

Taxonomy

Flavopunctelia (Krog) Hale, Mycotaxon 20(2): 682 (1984)

Flavopunctelia was established by Hale (1984); based on conidial and chemical characters and segregated it from *Punctelia* established previously by Krog (1982). *Flavopunctelia* was characterized by having bifusiform conidia and the presence of usnic acid (Thell & al. 2005). Conidium shape is considered an important character in genus identification (Krog 1982, Kärnefelt 1998). *Flavopunctelia* currently includes seven species. Divakar & Upreti (2003) stated that parmeliod lichens were widely distributed in different phytogeographical regions of India, that comprises approx. 11% of the total parmeliod lichens of the world. Based on morphotaxonomic studies, so far three *Flavopunctelia* species, *F. borrerioides*, *F. flaventior* and *F. soredica*, are recorded from India. Our study based on phylogenetic analyses coupled with morphological characters support the placement of *F. flaventior* and *F. soredica* in *Flavopunctelia*, *Parmotrema*-clade, *Parmeliaceae*.

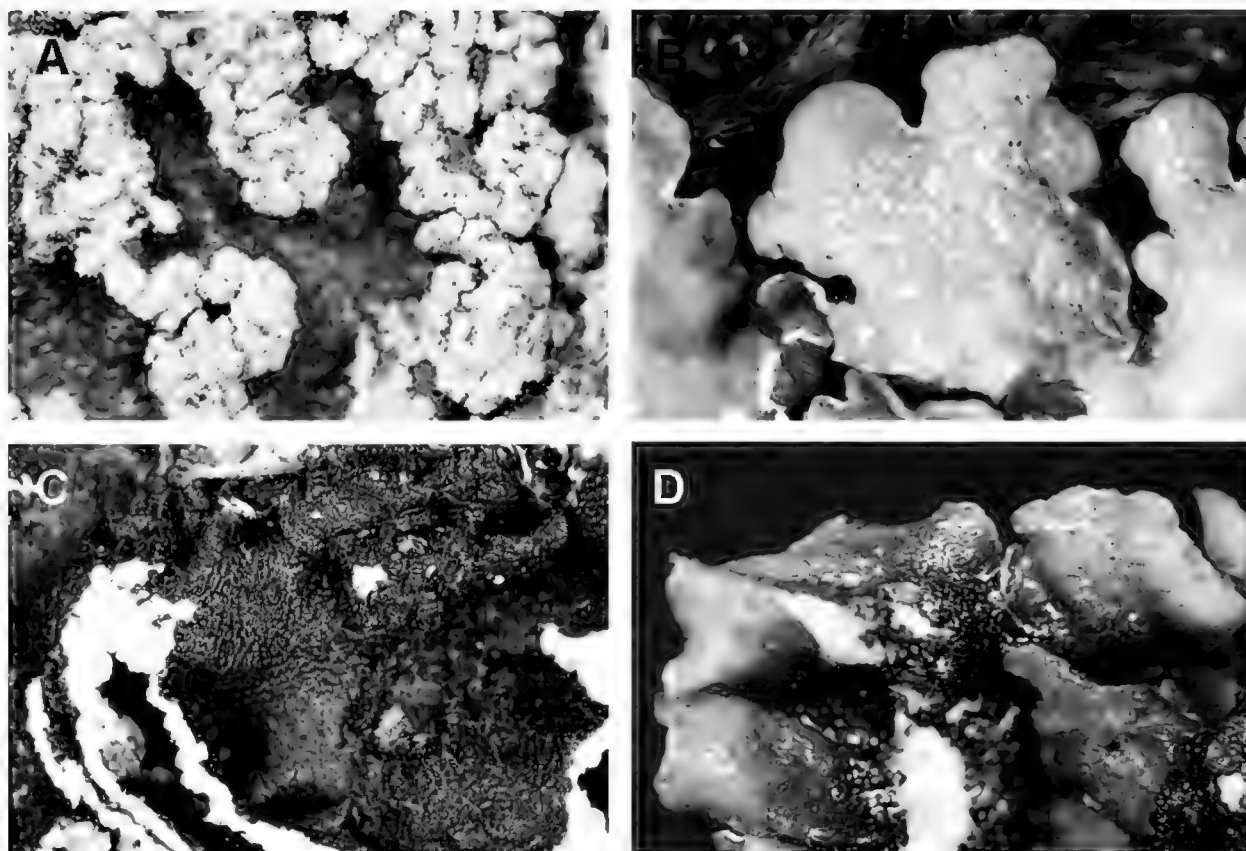***Flavopunctelia flaventior*** (Stirt.) Hale, Mycotaxon 20(2): 682 (1984) FIGS 3, 4

FIG. 3. *Flavopunctelia flaventior* (AMH 18.15(5)). 1, 2. Thallus; 3, 4. Lower surface and margin of thallus.
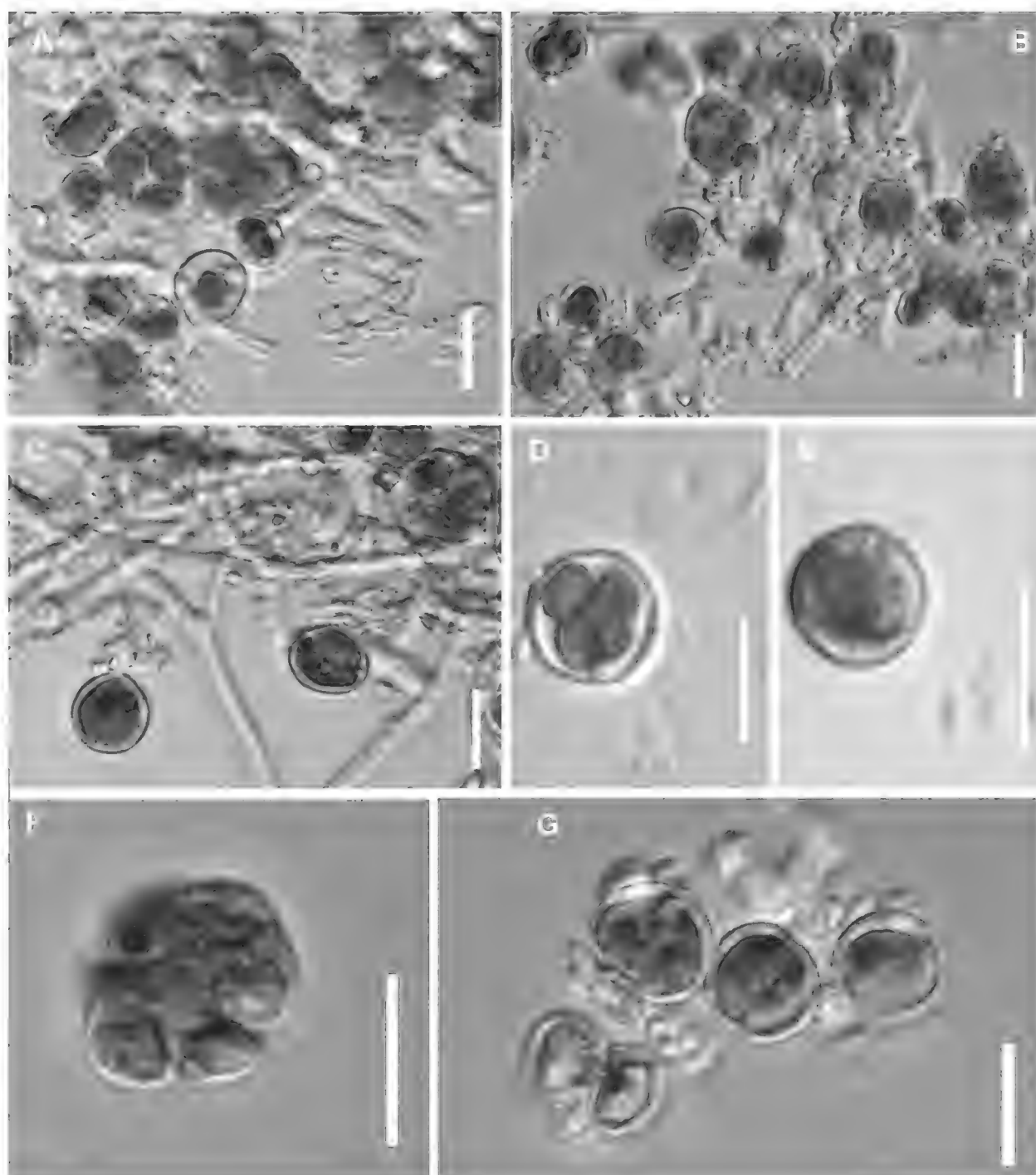
FIG. 4. *Trebouxia* sp., photobiont of *Flavopunctelia flaventior* (AMH 18.15(5)). 1, 3. Section of thallus; 4, 5. Mature vegetative cell; 6. Autosporangium; 7. Clusters of vegetative cells. Scale bars = 10 μm.

THALLUS, corticolous, yellowish green to greenish grey, closely adnate to the substratum, 5.0–8 cm across; LOBES sublinear to subirregular, imbricate 2.0–7.0 mm wide, plane; UPPER SURFACE rugulose or wrinkled in the centre, rarely reticulate; PSEUDOCYHELLAE white, round to slightly elongated; SOREDIA white, granular to farinose, round, marginal and densely distributed in the central part, laminal soralia arising from pseudocyhellae; MEDULLA

white; Lower surface black to pale brown, shiny towards margins; Rhizines sparsely distributed in the center, simple, short, concolorous with the lower surface. Apothecia not seen in sample examined.

Chemistry: cortex K+ yellow, KC–, C–; medulla C+ red; TLC: usnic acid (minor) and lecanoric acid (major)

Material examined—INDIA, Himachal Pradesh, Hamta Pass, 32.2708°N 77.3480°E, 23 August 2018, Bharati Sharma SF34 (AMH 18.15(5); GenBank MN006965, MN006966, MZ817004); SF33 (AMH 18.16(5); GenBank MN006962, MN006963, MZ817003); SF35 (AMH 18.17(5), GenBank MN536816, MN536903, MZ817005); SF36 (AMH 18.18(5); GenBank MN560036, MN560038, MZ817006); SF37 (AMH 18.26(5); GenBank MN567945, MN567948, MZ817007); SF44 (AMH 18.383(15); GenBank MN567946, MN567944); SF46 (AMH 18.385(15); GenBank MN562053, MN562055, MZ817008); SF66 (AMH 18.293(7); GenBank MN562194, MN562197, MZ817009).

Flavopunctelia soredica (Nyl.) Hale, Mycotaxon 20(2): 682 (1984)   Fig. 5
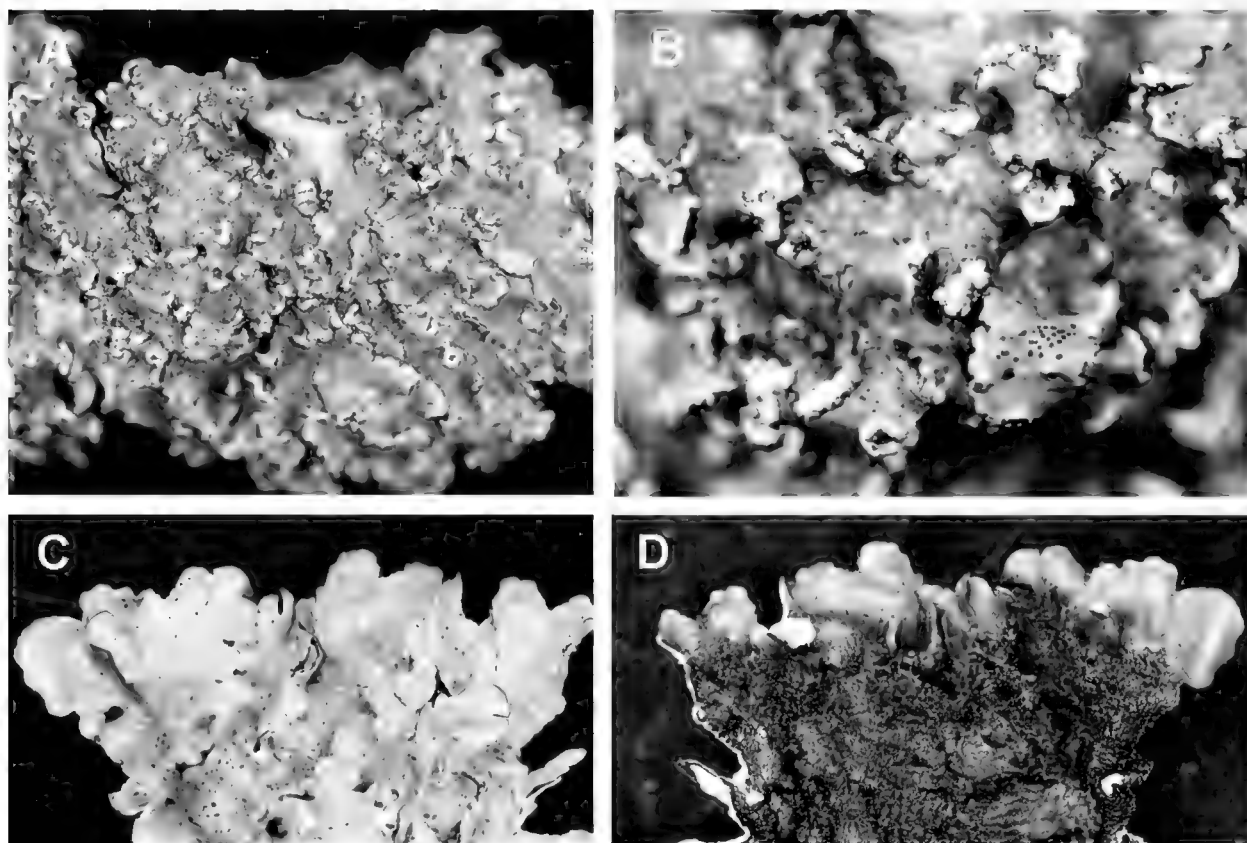

Fig. 5. *Flavopunctelia soredica* (AMH18.66(7)). 1–3. Thallus; 4. Lower surface and margin of thallus.

Thallus corticolous, upper surface yellowish green to greenish grey, up to 20 cm across; Lobes sub-irregular, imbricate to confluent 2.0–6.0 mm wide; Upper surface smooth to lacunose, rugulose or wrinkled longitudinally, pruinose near periphery; Pseudocyhellae absent; Soralia white, granular,

globose, marginal, crescent shaped; MEDULLA white; LOWER SURFACE black, erhizinate, shiny towards margins; Rhizines sparsely distributed in the center, simple, short, concolorous with the lower surface. APOTHECIA or PYCNIDIA not seen in sample examined.

CHEMISTRY: cortex K–; medulla K–, C+ red, KC+ red, P–; TLC: usnic acid and lecanoric acid

> MATERIAL EXAMINED—INDIA, HIMACHAL PRADESH, Hamta Pass, 32.2708°N 77.3480°E, 23 August 2018, Bharati Sharma SF64 (AMH 18.66(7); GenBank MN562090, MZ820402).

Punctelia Krog, Nord. J. Bot. 2(3): 290 (1982)

Punctelia is a segregate of *Parmelia* s.lat., characterized by unciform conidia and atranorin as major cortical substance. Sixty-three species of *Punctelia* have been reported with center of speciation in Africa and South America. Four *Punctelia* species have been reported from India: *P. borreri, P. neutralis* (Hale) Krog, *P. rudecta* (Ach.) Krog, and *P. subrudecta* (Nyl.) Krog. The phylogenetic analyses coupled with morphological characters support the placement of *P. borreri* in *Punctelia, Parmotrema*-clade, *Parmeliaceae.*

Punctelia borreri (Turner ex Sm.) Krog, Nord. J. Bot. 2(3): 291 (1982)   FIG. 6

THALLUS corticolous, 6–10 cm across; upper surface greenish grey to grey or bluish grey; LOBES often crowded, round, imbricate, ascending; upper surface smooth, rugulose, centrally shiny, sometimes partly pruinose; PSEUDOCYHELLAE present; small, punctiform, mostly near the margin; SORALIA laminal, clustered centrally, punctiform to confluent, linear; SOREDIA farinose, white to greyish white; MEDULLA white; LOWER SURFACE black, RHIZINES in the central part, simple, pale brown to black. APOTHECIA and PYCNIDIA not seen in sample examined.

CHEMISTRY: cortex K+ yellow; medulla K–, C+ red, KC+ red, P–; TLC: atranorin and gyrophoric acid

> MATERIAL EXAMINED—INDIA, HIMACHAL PRADESH, Hamta Pass, Curve 27, 32.2708°N 77.3480°E, 23 August 2018, Bharati Sharma SF67 (AMH 18.300(7); GenBank MN562210, MN562211, MZ817002); SF51 (AMH 18.141(14); GenBank MN947631, MN944564); SF48 (AMH 18.137(10); GenBank MN567947, MN567949, MZ817001).
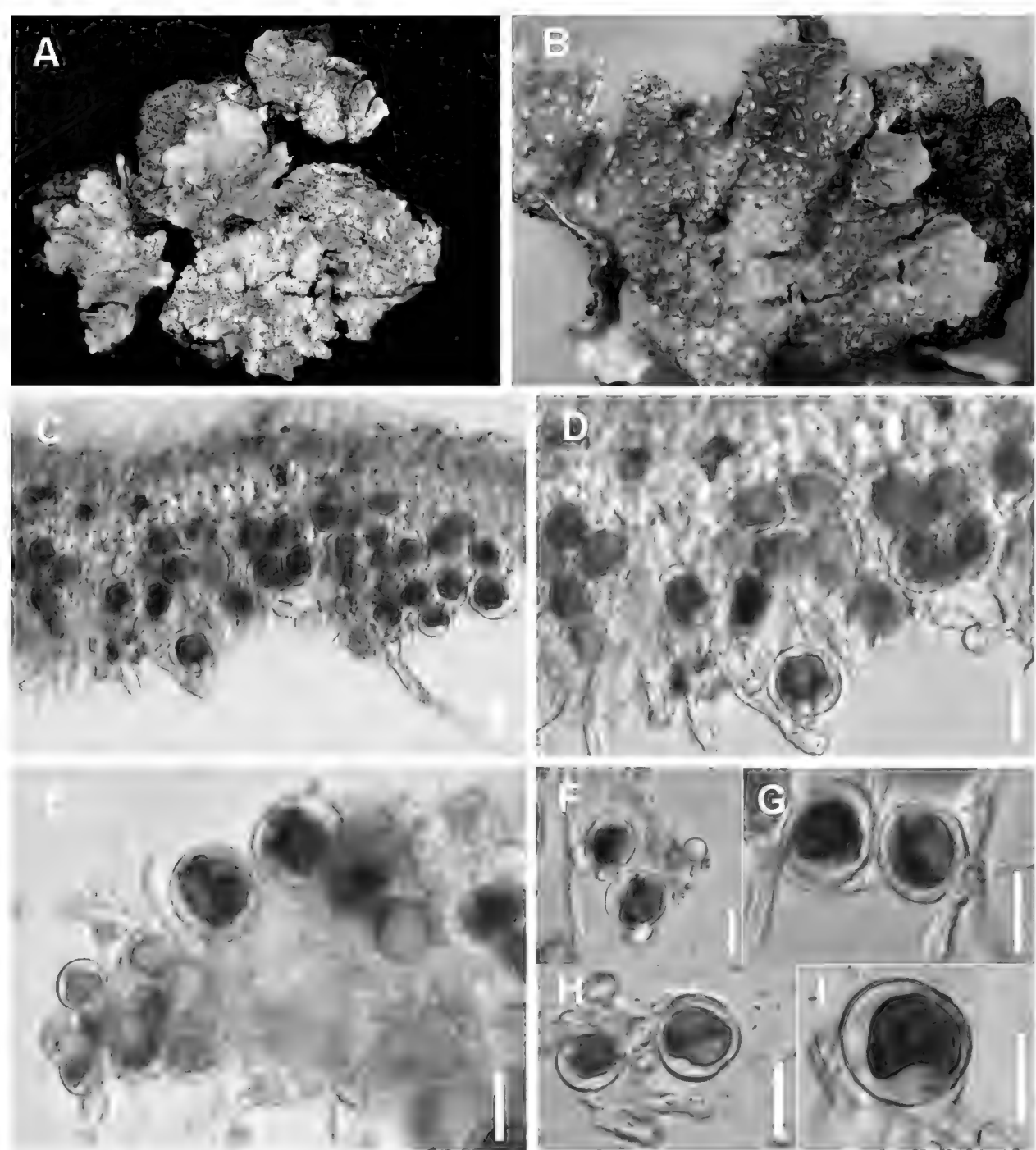
FIG. 6. *Punctelia borreri* (AMH 18.300(7)) and its photobiont symbiont *Trebouxia gelatinosa*. A. Thallus; B. Pseudocyphellate upper surface of thallus; C–E. Section of thallus; F–I. Mature vegetative cell. Scale bars = 10 μm.

Discussion

With the advent of molecular sequencing, generic concepts within the *Parmeliaceae* have been continually revised (Crespo & al. 2010, de Paz & al. 2011, Alors & al. 2016). The number of species known under this group has increased in the past decade by the inclusion of several newly discovered cryptic species some of which lack diagnostic morphological features to differentiate the species. Most of these phenotypically cryptic or semi-cryptic species need

urgent attention through DNA sequencing and phylogenetic analyses for their accurate identification (Divakar & al. 2010, 2015, 2017). As bioindicator species of air pollution and ecosystem assessment, understanding the diversity of the large family *Parmeliaceae* has an immense potential on climate change studies in the Himalayas. In our study, *Flavopunctelia* and *Punctelia* were identified based on morphology, chemotaxonomy, and molecular approaches. This is a holistic concept to taxonomize Indian *Parmeliaceae*; including mycobiont and phycobiont phylogeny to understand their diversity and symbiosis from different microhabitats of the Western Himalayas.

Phylogenetic analyses were used to inform the placement of three parmelioid species from the Indian Himalayas. The species of *Punctelia* (AMH 18.137(10), AMH 18.141(14), and AMH 18.300(7)) collected from the three different microhabitats of the Hamta pass from Pir Panjal ranges of Himachal Pradesh state aligned with the *Punctelia borreri* sequence (Hur 030736, GenBank DQ394373) and formed a well-supported clade (BS=87%, PP=0.96). *Punctelia borreri* was in a well-supported clade (BS=100%, PP=1) along with sister species *P. perreticulata* and *P. subpraesignis*. Distinguishing morphological characteristics such as the presence of pseudocyhellae and sorediate upper thallus surface and the presence of gyrophoric acid in the medullar region support the identity of the *Punctelia* species collected in this study as *P. borreri*.

Similarly, one of our *Flavopunctelia* specimens (AMH 18.66(7)) formed a well-supported clade (BS=99%, PP=1) with *F. soredica* (Aptroot no. 50612 (ABL)) originally collected and identified from New York Botanical Garden, USA.

Eight of our *Flavopunctelia* specimens aligned with Californian sequences of *F. flaventior* (Fig. 1: samples 17, MN006784; 18, MN006785; and 19, MN006786) forming a well-supported monophyletic clade adjacent to *F. soredica*. The present study evidences the wide distribution of *F. flaventior*, a flagship species widely distributed in different microhabitats of the Pir Panjal ranges of the Himalayas.

More sampling of *Flavopunctelia* and sequencing studies may be needed to resolve the complexity that exist in the authentication of material in the *F. flaventior* and *F. borrerioides* clade. The sole sequences of *F. borrerioides* (Thell & al. 2005) available in GenBank (AY773129) was not included in the study due to its paraphyletic placement within the *F. flaventior* clade. Further, our study revealed the existence of morphologically cryptic species in *F. flaventior* and *F. soredica* morphospecies, and additional studies will be needed to critically examine species boundaries.

The ITS sequence data and analyses enabled the identification of the photobionts as *Trebouxia* species in all the parmelioid species studied. The phylogenetic analyses revealed the existence of different *Trebouxia* species lineages in Indian *Flavopunctelia* and *Punctelia* collections. The photobiont of *P. borreri* was identified as *T. gelatinosa*. The photobionts of *F. flaventior* formed an exclusive clade of an apparently undescribed species of *Trebouxia*, with the photobiont of *F. soredica* also forming an unresolved basal clade to the *Trebouxia* species from *F. flaventior*. Our study revealed the diversity of *Trebouxia* species in *Flavopunctelia* and *Punctelia* samples collected from India and that may provide a baseline for further exploration of *Trebouxia* species diversity in Indian habitats. The data at hand allows us to conclude that *F. flaventior, F. soredica,* and *P. borreri* form associations with different *Trebouxia* species.

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New records of the genus *Cladonia* from Algeria

SARA BOUDIAF[1], MONIA ALI AHMED[1], RAQUEL PINO-BODAS[2,3*]

[1] *Laboratory of Plant Biology and Environment, Biology Department, Badji Mokhtar University, Annaba, 23000 Algeria*

[2] *Department of Biology and Geology, Physics and Inorganic Chemistry, Rey Juan Carlos University, 28933 Móstoles Madrid, Spain*

[3] *Royal Botanic Gardens, Kew, Richmond, TW9 3DS UK*

[*] CORRESPONDENCE TO: *rpino@ucm.es, raquel.pino@urjc.es*

ABSTRACT — Based on our studies of lichen-forming fungi in Kala National Park, Northeastern Algeria, four species, *C. cyathomorpha, C. dimorpha, C. monomorpha* and *C. subturgida*, were new reported for Algeria. Descriptions and taxonomic notes are provided; and a key is presented for all the *Cladonia* species known from Algeria.

KEY WORDS—*Cladoniaceae, Lecanorales*, Mediterranean Region, taxonomy

Introduction

The Mediterranean basin has been recognized as a global biodiversity hotspot (Médail & Quézel 1997). The diversity of lichen-forming fungi is well known in some Mediterranean countries, such as France (Ozenda & Clauzade 1970, Roux & al. 2014, 2017), Italy (Nimis 1993, 2016, Nimis & Martellos 2020, Nimis & al. 2018), Greece (Abbott 2009, Linda in Arcadia 2022), and Spain and Portugal (Llimona & Hladún 2001). However our knowledge of the diversity of lichen-forming fungi in North African countries is still poor.

Algeria is located in the Maghreb, bordered to the north by the Mediterranean Sea, to the east by Tunisia and Libya, to the southeast by Niger, to the southwest by Mali and Mauritania, to the west by Morocco and Western Sahara. It is the second largest country (2.4 million km²) from Africa. Four fifths of the territory are occupied by the Sahara desert. In the north of the country two

parallel mountain ranges, Tellian Atlas and High Plateau, form a natural barrier with the Sahara. In these regions, pine, cork oak, holm oak, carob forests, and a large number of shrubs, are the dominant vegetation.

Approximately 1100 taxa of lichen-forming fungi have been reported from Algeria (Amrani & al. 2018). Although in recent years several studies of lichen diversity have been carried out in certain protected areas (Rebbas & al. 2011, Serradj & al. 2013, Ali Ahmed & al. 2018), large areas of the country have not yet been explored, due to the scarcity of specialists in lichenology.

This study was carried out in the region of El Kala, considered as an Important Plant Zone (API) of Northeast Algeria, with a remarkable composition of rare, threatened, or endemic plants (Radford & al. 2011). It also offers an exceptional lichen flora. In the present study, we focus on *Cladonia*, a genus of lichen-forming fungi whose species are characterized by a dimorphic thallus, composed of a crustose or squamulose primary thallus and a fruticose secondary thallus. Most of the species are terricolous, growing on open areas with acid soils, although some develop on calcareous soils, and bases and trunks of trees (Ahti 2000, Ahti & Stenroos 2013, Burgaz & al. 2020). Twenty-five species of *Cladonia* have been reported from Algeria (Amrani & al. 2018), eight of them from the Edough Peninsula in Northeast Algeria (Ali Ahmed & al. 2018).

The aim of this paper is to report four new *Cladonia* species from Algeria, *C. cyathomorpha, C. dimorpha, C. monomorpha* and *C. subturgida*, and to put forward a key for all the *Cladonia* species reported from the country.

Materials & methods

Study Region

The specimens were collected between 2018 and 2019 from the El Kala National Park, in dune and mountain stations. This park is located in Northeast Algeria, at 36.71–36.95°N 7.72–8.62°E, with an altitude range from sea level to 1189 m. The park is limited to the north by a cordon of dunes bordering the Mediterranean Sea, to the east by the Algerian-Tunisian border, to the south by the mountains of Medjerda and to the west by the town of El Tarf and the vast marshes of Mekkada (Loukkas 2006). The region is characterized by a humid bioclimate, with average annual rainfall of 910 mm, and average annual minimum and maximum temperatures of 9°C and 30°C (Sarri & al. 2014).

Woodlands cover 70% of the total area of the park, including *Quercus canariensis, Q. coccifera, Q. suber, Pinus pinaster, P. halepensis,* and *Alnus.* Some areas are dominated by introduced species such as *Acacia mearnsii* and *Eucalyptus globulus.*

Morphological and chemical studies

The specimens were identified based on morphological characters (James 2009, Burgaz & Ahti 1992, 1994; Burgaz & al. 2020, Ahti 2000, Ahti & Hammer 2002, Pino-Bodas & al. 2012a, 2020, Ahti & Stenroos 2013). To confirm the identification of *C. subturgida,* ITS rDNA was sequenced using the protocols described in Pino-Bodas & al. (2020). The new sequence has been deposited in GenBank.

The descriptions of the species were based on: 10 specimens of *C. dimorpha*; 2 of *C. subturgida*; 1 of *C. cyathomorpha*; and 1 of *C. monomorpha*. The specimens were studied under stereomicroscope Leica EZ6 and Nikon Eclipse E400 microscope. Measurements were taken of length, width, and thickness of squamules and of the width, length, and thickness of podetia. Three squamules and three to five podetia per specimen were studied. Hand-cut sections of the squamules and podetia were made to study the microscopical features. Measurements of cortex, algal layer, medulla, stereome, and size of soredia were taken at 400×. Hand-cut sections of apothecia were taken to study and measure the ascospores at 1000×. In the text the measurements are indicated as (minimum–){X − SD}–{X + SD}(–maximum), where X is the arithmetic mean and SD the corresponding standard deviation, followed by the number of measurements. All the specimens are deposited in the Herbarium, Biology Department, Badji Mokhtar University, Annaba, Algeria (AAM), and a few duplicates at in the Mycology Herbarium, Royal Botanic Gardens, Kew, UK (K-M) or at the Herbarium, Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense de Madrid, Spain (MACB).

The secondary metabolites were studied using C, K, and Pd spot tests and by thin-layer chromatography (TLC) according to standardized procedures (White & James 1985, Orange & al. 2001), with solvent systems A and C.

Taxonomy

Cladonia cyathomorpha Stirt. ex Walt. Watson, J. Bot. 73: 156, 1935.   FIG. 1A,D

PRIMARY THALLUS: squamulose, persistent, squamules 3–6 mm long × 1–3 mm wide (N = 3), margins rolled up and becoming more or less erect when dry; upper side grey to greenish, lower side white to dirty white, more or less veined, veins light to dark brown. ANATOMY OF SQUAMULES: (72–)79.16–101.19(–108) µm thick; cortex (19.2–)22.54–32.84(–36) µm thick; algal layer (16.8–)20.99–29.33(–31) µm thick; medulla (26.4–)29.88–45.18(–48) µm (N = 3). PODETIA: not very frequent, 4–6 mm tall, (0.41–)0.40–0.81(–0.97) mm wide (N = 3), scyphose, grey to greenish, surface smoothly corticate at the base, densely covered with small granules inside the scyphi, granules size (19.2–)24.71–52.53(–60) µm diameter (N = 22), occasionally scyphi with marginal proliferations. PODETIAL WALL: (117.66–)143.37–203.85(–226.7) µm; cortex (16.8–)21.98–37.72(–41.5) µm; algal layer (27–)34.65–57.25(–60.9) µm;
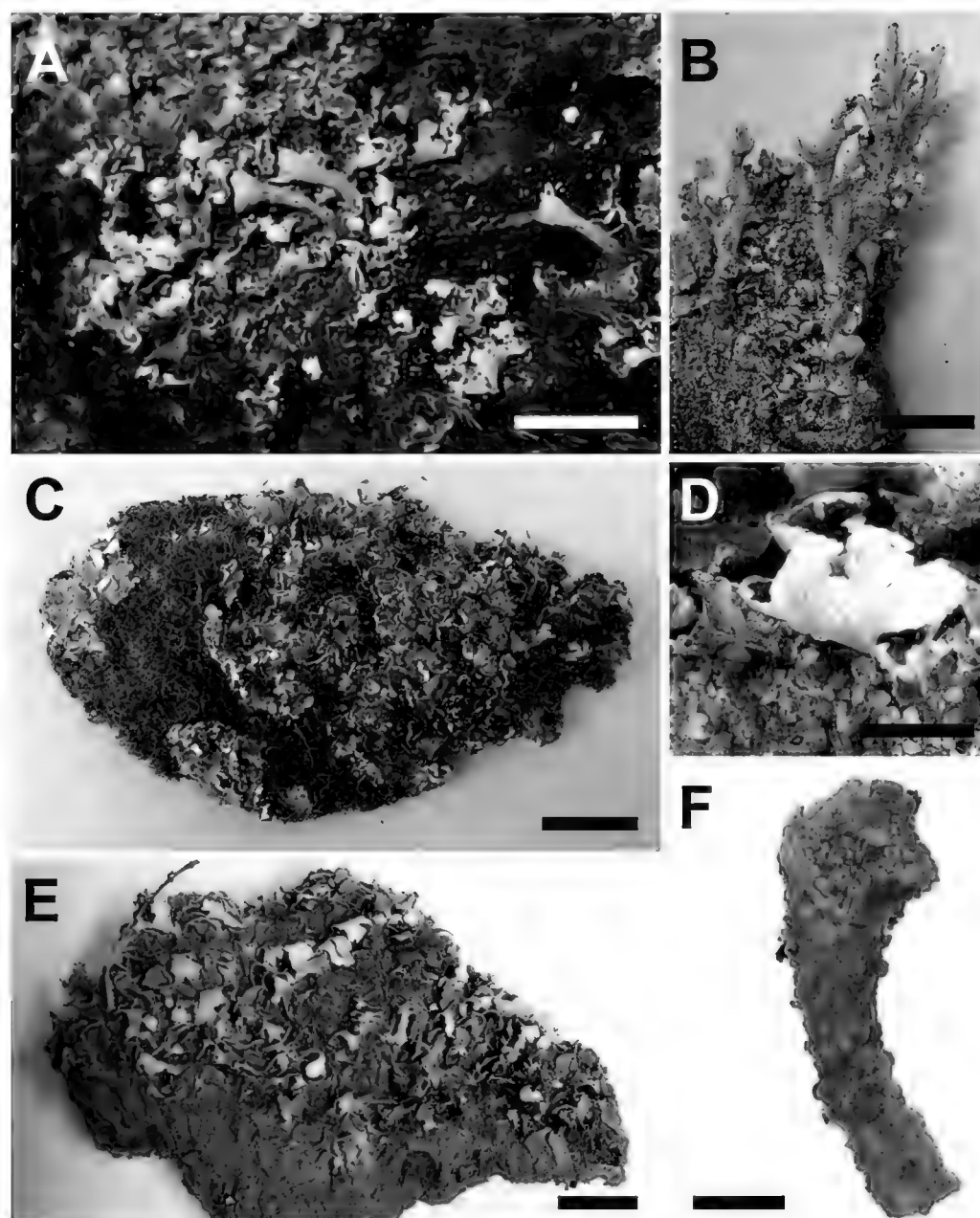
Fig. 1. *Cladonia cyathomorpha* (AAM-408): A. Thallus; D. Veins in the lower side of squamule. *Cladonia dimorpha* (AAM-371): B. Thallus. *Cladonia monomorpha* (AAM-179): C. Thallus; F. Podetium. *Cladonia subturgida* (AAM-409): E. Thallus. Scale bars: A, B = 5 mm; C, E = 10 mm; D, F = 1 mm.

medulla (44–)52.53–99.43(–112) μm; stereome (9.6–)13.7–29.96(–36) μm (N = 3). Apothecia: very rare, brown, on the scyphal margin, ascospores simple, fusiform to ellipsoid, hyaline (4.8–)6.19–10.66(–11.52) μm long (2.88–)3.13–5.31(–5.76) μm wide (N = 10). Pycnidia: frequent on margins of scyphi, conidia not observed.

Chemistry— C–, K–, Pd + red. Fumarprotocetraric acid complex.

Habitat and distribution—This species is not frequent in Algeria. Only one specimen has been found, growing on a tree trunk, in *Pinus pinaster* woodland,

at 4 m altitude. It is distributed in Western Europe, frequent in Mediterranean countries (Burgaz & al. 2020), reported also in North Europe, Macaronesia, and Argentina (Ahti & Stenroos 2013).

REMARKS—This is a new record for Algeria. It can be mistaken for *Cladonia humilis*, *C. pyxidata*, and *C. chlorophaea*, also reported in Algeria. *Cladonia humilis*, has shorter squamules, without veins or less marked, and farinose soredia. The primary thallus of *C. pyxidata* lacks veins in the lower side and has corticate plates inside the scyphi. *Cladonia chlorophaea* has corticate podetia with granulose soredia on the scyphi. The squamules of our specimen are slightly narrower than those reported by Burgaz & al. (2020), 5–8 mm wide, being similar in the rest of the characters.

SPECIMEN EXAMINED—**ALGERIA. OUM TBOUL PROVINCE: National Park El Kala,** Mechta Tonga, 36.8884°N, 8.4483°E, 4 m, sandy soil, 2.03.2019, M. Ali Ahmed & S. Boudiaf 408 (AAM, K-M 1434284).

Cladonia dimorpha S. Hammer, Mycotaxon 37: 339, 1990.   FIG. 1B

PRIMARY THALLUS: squamulose, persistent, squamules 4–6 mm long × 3–4 mm wide (N = 9), lobate and slightly dentate, rarely with black rhizines at margins, sometimes sorediate in the lower side. ANATOMY OF SQUAMULES: (100.8–)122.45–220.91(–285.4) μm thick; cortex (24–)27.91–50.09(–55.2) μm thick; algal layer (12–)16.67–69.07(–91.2) μm thick; medulla (36–)51.86–143(–156) μm (N = 9). PODETIA: frequent, ashy white to green, 3–16 mm tall, (0.39–)0.50–1.11(–1.31) mm (N = 9) wide at base, scyphose, scyphi 2–6 mm diam., always with branching proliferation at the margins. Surface smoothly corticate, cortex extending from the base to scyphi, sometimes with small squamules, sorediate at the scyphi (12–)14.18–25.16(–28.8) μm diameter (N = 51), and within scyphi. PODETIAL WALL: (115.2–)120.84–218.96(–252) μm; cortex (14.4–)29.55–66.81(–69.8) μm; algal layer (7.2–)11.95–45.65(–55.2) μm; medulla (40.8–)33.83–119.07(–144) μm; stereome (12–)10.74–18.06(–21.6) μm (N = 9). APOTHECIA: at tips of proliferation, brown or dark brown, ascospores hyaline, simple, oblong (8.64–)9.02–14.19(–16.32) μm long (2.88–)3.11–4.93(–5.76) μm wide (N = 25). PYCNIDIA: common, at the scyphal margins, globose and black, conidia not observed .

CHEMISTRY—Two chemotypes have been found. Chemotype I: C–, K–, Pd + red. Furmaprotocetraric acid complex, chemotype II: C–, K+ yellow, Pd+ red. Furmaprotocetraric acid complex, and atranorin. Chemotype I is the commoner.

Habitat and distribution—This species is not common in Algeria, growing on clayey and sandy soils, tree trunks and on mosses, in *Quercus suber*, *Pinus pinaster* woodlands, and *Eucalyptus globulus* plantation. It was collected in 3 localities in the National Park El Kala from sea level to 34 m altitude. The distribution of this species is not well-known, it has been reported from Western North America, Western Europe and Macaronesia (Pino-Bodas & al. 2017, Burgaz & al. 2020).

Remarks—*Cladonia dimorpha* is similar in habit to *C. pyxidata* and *C. humilis*, but it has long, fissured marginal proliferations, similar to fertile podetia of *C. furcata*. When the proliferations are not developed (in juvenile podetia) the species is very difficult to identify (Ahti & Hammer 2002, Pino-Bodas & al. 2017). The identity of this species is uncertain in Europe, and Burgaz & al. (2020) considered than more studies are necessary to solve its taxonomic status. Our material is similar to prior published description (Burgaz & Ahti 2009).

> Specimens examined—**ALGERIA. Oum Tboul province:** National Park El Kala. Mechta Tonga, 36.8884°N, 8.4483°E, 4 m, sandy soil, 2.03.2019, M. Ali Ahmed & S. Boudiaf 370 (K-M 1434285), 371, 372, 373 (AAM) [Chemotype II]; **Brabtia province. Gantra Hamra**, 36.8642°N 8.3467°E, 34 m, clayey silt soil, 8.12.2018, M. Ali Ahmed & S. Boudiaf 254 (K-M 1434286), 255, 256, 257 (AAM) [Chemotype I]; **Cap Rosa**, 36.9491°N 8.2401°E, 10 m, clayey silt soil, 10.10.2019, M. Ali Ahmed & S. Boudiaf 632 (AAM), 633 (MACB 116475) [Chemotype I].

Cladonia monomorpha Aptroot, Sipman & Herk, Lichenologist 33: 273, 2001.

Fig. 1c,f

Primary thallus: squamulose, persistent, squamules well developed, 2–5 mm long × 1–3 mm wide (N = 3), lobate, not much incised, erect but usually narrowly recurved at margin, upper side olive green, lower side white. Anatomy of squamules: (50.4–)58.84–162.64(–168) μm thick; cortex (9.6)10.99–45.23(–52.8) μm thick; algal layer (9.6–)11.6–39.14(–48) μm thick; medulla (21.6–)22.63–91.87(–98.4) μm (N = 3). Podetia: small 2–4 mm tall, (0.31)0.35–0.47(–0.51) mm (N = 3) wide at base, scyphose, scyphi simple without marginal proliferations, green to brown, surface mostly corticate with bullate plates in the upper part and inside the scyphi. Podetial wall: (122.4–)123.89–168.91(–172.8) μm; cortex (40.8–)41.08–60.68(–62.4) μm; algal layer (21.6–)24.51–36.93(–38.4) μm; medulla (28.8–)33.97–57.23(–60) μm; stereome (7.2–)10.73–25.75(–26.4) μm (N = 3). Apothecia: not observed. Pycnidia: not common at the margin of the scyphus, dark brown. Conidia not observed.

CHEMISTRY— C–, K–, Pd+ red. Fumarprotocetraric acid complex.

HABITAT AND DISTRIBUTION—This is found on clayey silt soil in *Erica arborea* and *Calicotome villosa* shrubland, at 34 m altitude. This species has been previously reported from several Mediterranean countries and it is widespread in Europe (Aptroot & al. 2001, Burgaz & Ahti 2009).

REMARKS—The taxonomical status of C. *monomorpha* is controversial. Some authors consider it as a morphological variation of *C. pyxidata* (Ahti & Stenroos 2013, Burgaz & al. 2020), but according to Kowalewska & al. (2008) it is very distinct and deserves species level recognition. It can be distinguished from *Cladonia pyxidata* by the presence of bubbled and unflattened plates (Aptroot & al. 2001, Burgaz & al. 2020). The phylogenetic studies of *Cladonia* (Stenroos & al. 2019) showed that C. *pyxidata* complex needs more study. The specimen examined differ from the protologue (Aptroot & al. 2001) in having shorter podetia (up to 3 cm high in the original description).

> SPECIMEN EXAMINED— **ALGERIA. BRABTIA PROVINCE, National Park El Kala,** Gantra Hamra, 36.8642°N 8.3467°E, 34 m, clayey silt soil, 8.12.2018, M. Ali Ahmed & S. Boudiaf 179 (AAM, K-M 1434287).

Cladonia subturgida Samp., Ann. Sci. Acad. Polytecn. Porto 13: 106, 1918.

FIG. 1E

PRIMARY THALLUS: squamulose, persistent, squamules large 2–9 mm long × 1–4 mm wide (N = 6), deeply lobed, upper side dark green to olivaceous, lower side white. ANATOMY OF SQUAMULES: (112.8–)115.06–188.02(–216) μm thick; cortex (21.6–)23.98–41.5(–48) μm thick; algal layer (12–)17.69–39.21(–40.8) μm thick; medulla (55.2–)63.41–117.27(–134.4) μm (N = 6). PODETIA: not observed. PYCNIDIA: black, frequent on upper side of the squamules, conidia not observed.

CHEMISTRY— C–, K+ yellow, Pd –. Atranorin and protolichesterinic acid.

HABITAT AND DISTRIBUTION— This is a terricolous species growing on clayey silt soil in a woodland of *Quercus suber* with *Pinus pinaster, Erica arborea, Calicotome villosa, Phillyrea angustifolia,* and *Helianthemum nummularium* at 100 m altitude. This species is known from Mediterranean and Macaronesian Regions (Kocakaya & al. 2018; Pino-Bodas & al. 2020).

REMARKS—This species has probably been overlooked. Although it has been found only in one locality, we think that it is widely distributed in Northern Algeria, since a recent study proved that C. *subturgida* is a common species in

the Mediterranean region (Pino-Bodas & al. 2020). Our identification has been confirmed by ITS rDNA.

It can be mistaken for *Cladonia firma* and *C. cervicornis*, other species with dominant primary thallus. *Cladonia cervicornis* has larger and sharply recurved squamules, brownish green on the upper side and pinkish brown on the underside, and generally contains only furmarprotocetraric acid. *Cladonia firma* has also thicker and larger squamules and the olive green color of the upper side usually with whitish apices, greyish brown in the lower side (Burgaz & al. 2020). The specimen fits to previous descriptions (Pino-Bodas & al. 2012b; Burgaz & al. 2020).

Specimen examined— **ALGERIA. Oum Tboul province, National Park El Kala**, Haddada, 36.895752°N 8.617074°E, 100 m, clayey silt soil, 2.03.2019, M. Ali Ahmed & S. Boudiaf 409, 410 (AAM; Genbank OQ267627).

Key to *Cladonia* species of Algeria

1. Podetia richly branched, without cortex 2
1. Podetia absent or not richly branched or, if branched, with cortex 3
2. Branching pattern mainly dichotomous, UV+ white .... *C. mediterranea*
2. Branching pattern mainly trichotomous, UV− .... *C. arbuscula*
3. Squamules of primary thallus dominant, with or without podetia 4
3. Podetia dominant, primary thallus persistent or evanescent but never dominant 12
4. Growing on calcareous soil 5
4. Growing on acid or neutral soil or on wood 7
5. Squamules imbricate, forming a rosette .... *C. pocillum*
5. Squamules scattered 6
6. Squamules small <4 mm long .... *C. cariosa*
6. Squamules >4 mm long .... *C. symphycarpa*
7. Squamules yellowish in the lower side .... *C. foliacea*
7. Squamules with the lower side white, brownish or grey 8
8. Squamules Pd− or + yellow 9
8. Squamules Pd+ red 10
9. Squamules with coralloid margin, Pd + yellow .... *C. parasitica*
9. Squamules without coralloid margin, fragile, Pd− .... *C. subturgida*
10. K+ yellow, with pruinose margins .... *C. firma*
10. K−, margins without pruina 11
11. Squamules small, with crenulate margin, lower side white .... *C. caespiticia*
11. Squamules >5 mm, with entire margin, lower side brownish to grey
.... *C. cervicornis*
12. Podetia with scyphi 13
12. Podetia without scyphi 28
13. Podetia completely corticate 14

13. Podetia sorediate, granulose, squamulose or partially corticate 16

14. Podetia slender, >20 mm long, with narrow scyphi, some tips without scyphi, with lateral proliferations .... *C. gracilis*

14. Podetia robust, with wide scyphi, with central proliferations 15

15. Podetia with more than 1 proliferation per tier, 1–4 tiers .... *C. cervicornis*

15. Podetia with a single proliferation per tier, 1–7 tiers .... *C. verticillata*

16. Podetia farinose sorediate 17

16. Podetia with granules, squamules, corticated plates or discontinuous cortex 25

17. Podetia slender, with narrow scyphi 18

17. Podetia short, with wide scyphi 19

18. Podetia unbranched, greenish with regular scyphi .... *C. coniocraea*

18. Podetia simple or branched, whitish, with irregular scyphi and frequently marginal proliferations .... *C. subulata*

19. Podetia mainly sorediate, with cortex restricted to the base .... *C. fimbriata*

19. Podetia with cortex reaches the scyphal margin and soredia or granules in the upper part 20

20. UV+ blue .... *C. grayi*

20. UV– 21

21. Scyphi with farinose soredia, <50 μm 22

21. Scyphi with granulose soredia, >50 μm 24

22. Podetia with marginal proliferations, proliferations longitudinally split
.... *C. dimorpha*

22. Podetia without marginal proliferations 23

23. Podetia with long stalk, K– .... *C. conista*

23. Podetia without stalk, K+, rarely K– .... *C. humilis*

24. Squamules with veins in the lower side .... *C. cyathomorpha*

24. Squamules without veins in the lower side .... *C. chlorophaea*

25. Podetia with discontinuous cortex and frequently with squamules ..... *C. ramulosa*

25. Podetia with bullate or flat plates on scyphi 26

26. Podetia with flat plates on scyphi .... *C. pyxidata*

26. Podetia with bullate plates on scyphi 27

27. Squamules ± erect, scattered .... *C. monomorpha*

27. Squamules imbricate, forming rosettes .... *C. pocillum*

28. Podetial surface covered by numerous squamules 29

28. Podetial surface corticate or sorediate or with scattered squamules 32

29. UV+ White, podetia with open apex, forming funnels .... *C. squamosa*

29. UV–, podetia with funnels or without funnels 30

30. Pd+ yellow, containing thamnolic acid 31

30. Pd+ red, containing fumarprotocetraric acid complex .... *C. scabriuscula*

31. Podetia small, coralloid .... *C. parasitica*

31. Podetia up to 50 mm, without coralloid appearance .... *C. squamosa*

32. Podetia sorediate, with cortex restricted to basal part 33

32. Podetia completely corticate or partialy decorticate but never sorediate 35

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Erysiphe bistortae sp. nov. on *Bistorta amplexicaulis* from Pakistan

Irsa Zafar[1]*, Najam ul Sehar Afshan[1], Javeria majeed[1],
Abdul Rehman Niazi[1], Abdul Nasir Khalid[1]

[1] *Institute of Botany, University of the Punjab,*
    Quaid-e-Azam Campus, Lahore, 54590, Pakistan
* Correspondence to: *irsazafar2@gmail.com*

Abstract—A powdery mildew fungus appearing on the leaves of *Bistorta amplexicaulis* in Pakistan is described as a new species, *Erysiphe bistortae*. This species is characterized by its large-sized chasmothecia and relatively small conidiophores. Because of biological and morphological differences from related species, supplemented by results of molecular sequence analysis, the Asian powdery mildew on *B. amplexicaulis* warrants description as a species of its own.

Key words— *Erysiphaceae, Polygonaceae*, taxonomy

Introduction

Polygonaceae is a cosmopolitan family comprising roughly 12,000 species and 48 genera (Sanchez & Kron 2008). They are an assemblage of morphologically diverse trees, shrubs and herbs (Hutchinson & Dalziel 1954; Brummitt 1992). Their distribution is worldwide from the tropics to the arctic, yet a large proportion of the species are concentrated in the northern temperate region (Heywood 1978). This family is represented in Pakistan by 19 genera and 103 species, with the genus *Bistorta* (L.) Scop. represented by seven species (Qaisar 2001), including *B. amplexicaulis* (D. Don) Greene. *Bistorta amplexicaulis* is a perennial shrub, with a Pakistani distribution in Chitral, Swat, Kashmir, and Murree Hills (Nasir & al. 1972). Its leaves are used for making tea which is exceptionally effective for curing fever, flu, and aching joints (Qureshi & al. 2007). During a survey of powdery mildews in Azad Jammu & Kashmir and

Khyber Pakhtunkhwa, Pakistan, this economically important plant was found to be infected with a species of the genus *Erysiphe*.

Previously, only one powdery mildew species, *Erysiphe polygoni* DC., has been reported to occur on *Bistorta amplexicaulis* in Pakistan (Ahmad 1978). After detailed analysis, our newly collected specimens were found to be different from previously reported species of *Erysiphe*. This species is described morphologically along with molecular analysis, which support its identification and proposal as a new species, *Erysiphe bistortae*.

Materials & methods

Collection & Preservation

Leaves infected with powdery mildew were collected during the phytopathological survey of Azad Jammu & Kashmir and Khyber Pakhtunkhwa, Pakistan, in 2020. The study areas lie in the north-eastern part of Pakistan that is characterized by green, rough and undulating terrain and is floristically very rich (Dar 2012). For preservation, the infected plants were shade dried with blotting paper and further protected in envelopes. The specimens are deposited at the Herbarium of Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Morphological characterization

Infected samples were examined macromorphologically under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan) and slides were prepared in lactic acid. The micromorphology of hyphae on the host, hyphal appressoria, conidia, conidiophores, shapes and sizes of chasmothecia, asci, and ascospores were examined under a compound microscope (SWIFT M4000-D) with a 9MP camera system. A minimum of twenty measurements were taken for each diagnostic feature.

DNA extraction, PCR amplification, phylogenetic analysis

Dried powdery mildew colonies were scratched off with the help of razor blades from fresh fungal specimens, ground in liquid nitrogen and stored in Eppendorf tubes at –18°C. DNA was extracted using Thermo Scientific Gene JET Plant Genomic DNA Purification Mini Kit #K0791. The Internal Transcribed Spacer (ITS) region was amplified using PMITS1/PMITS2 primers (Cunnington & al. 2003). PCR products were visualized on 1% agarose gel with ethidium bromide through Gel documentation system (Sambrook & Russel 2001). PCR products were sent for sequencing to Tsingke, China. By using BioEdit, raw sequenced data were edited (Hall 1999). The ITS sequences were BLAST searched against the GenBank database (www.ncbi.nlm.nih.gov). Maximum percent identity and query coverage of sequences with related taxa were noted. All sequences along with the new sequences were aligned through MAFFT (Multiple sequence alignment tool). Sequences were aligned and trimmed at conserved sites from both 5' / 3' ends. The phylogenetic tree was executed within MEGA 6.0 (Tamura & al. 2013), using Maximum Likelihood Method based on

TABLE 1. Strains and sequences used in the phylogenetic analyses of *Erysiphe* species. New sequences are in bold.

SPECIES	COUNTRY	VOUCHER	GENBANK (ITS)
E. alphitoides	United Kingdom	OE2015PMCS301	KY660893
	United Kingdom	RHS287283	KP686268
E. berberidicola	South Korea	KUS-F30268	MG938640
	South Korea	KUS-F30028	MG938639
E. betae	Greece	Se1	KY399969
	China	ZKEB001A	KF268348
	Korea	KUS: F29140	KX574674
	Pakistan	AA#4 (PUP Bot.01	MN368297
E. bistortae	**Pakistan**	**LAH36943**	**MZ048848**
	Pakistan	**LAH36143**	**MZ048849**
E. castaneigena	Korea	KUS:F28761	KY926846
	Korea	KUS:F28676	KY926844
	Korea	KUS:F28682	KY926845
E. cruciferarum	United Kingdom	**OE2015PMCS236**	KY660884
	United Kingdom	**OE2015PMCS250**	KY660924
E. diffusa	Spain	Erd1	MK673961
E. euonymicola	United Kingdom	OE2016PMCS1	KY661150
E. heraclei	Argentina	MUMH<JPN>:1874	LC010004
	Korea	KUS-F27279	KF111807
	China	HMNWAFU-CF2010265	KR048065
	Australia	BRIP 68828	MT174194
	Australia	BRIP 59438	MT174193
	Korea	KUS:F28393	KP729443
	United Kingdom	OE2015PMCS211	KY660878
	United Kingdom	OE2016PMCS5	KY661135
E. intermedia	United Kingdom	OE2014PM79CS	KY660797
	United Kingdom	OE2015PMCS297	KY660904
E. limonii	USA	490P06380636	MK094072

Species	Country	Voucher	GenBank (ITS)
E. ludens	United Kingdom	OE2015PMCS298	KY660905
	United Kingdom	OE2015PMCS259	KY660901
E. malvae	Ukraine	MUMH\<JPN\>:2570	LC010041
	Ukraine	MUMH\<JPN\>:2569	LC010040
E. mori	China	Ma001	KY910120
E. pisi	United Kingdom	OE2013PM18	KY660750
	United Kingdom	OE2016PMCS80	KY653209
E. polygoni	Azerbaijan	MUMH\<JPN\>:7036	LC328322
	Azerbaijan	MUMH\<JPN\>:7045	LC328323
	China	PM	MW169023
	—	—	AF011307
	United Kingdom	OE2016PMCS63	KY661154
E. trifoliorum	United Kingdom	OE2015PMCS231	KY660892
	United Kingdom	OE2015PMCS265	KY660909
E. tortilis	United Kingdom	OE2014PM83CS	KY660755

Kimura 2-parameter with 1000 rapid bootstrap replicates. The model of evolution was selected by searching for the best DNA model for ML analysis in MEGA 6.0 (Tamura & al. 2013).

Results

Taxonomy

Erysiphe bistortae Afshan, I. Zafar & Khalid, **sp. nov.**   Figs 1, 2
MB839866

> *Erysiphe bistortae* differs from *Erysiphe polygoni* by its larger chasmothecia; and from *E. betae*, *E. heraclei*, and *E. malvae* by its conidiophores having relatively short foot cells followed by 1–2 shorter cells.
>
> Type—Pakistan, Azad Jammu & Kashmir, Las Dana, 33.92°N 73.96°E, 2500 m alt., on leaves of *Bistorta amplexicaulis* (D. Don) Greene (*Polygonaceae*), 26 Sep. 2020, N.S. Afshan and I. Zafar LDH-01 (**Holotype**, LAH36943; GenBank MZ048848).
>
> Etymology—the species epithet refers to the host genus, *Bistorta*.
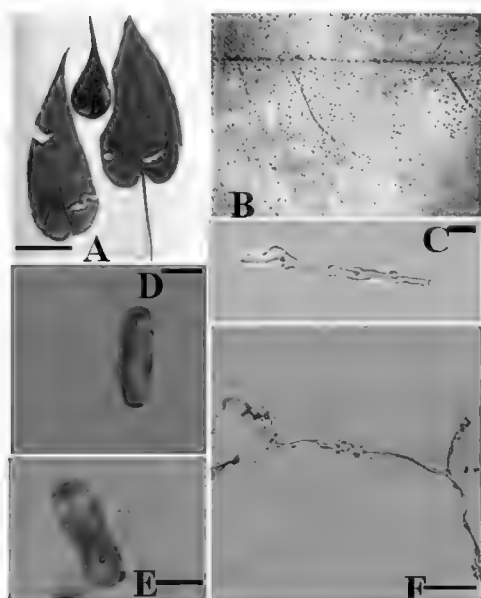
Fig. 1. *Erysiphe bistortae* (holotype, LAH 36943). A. Infected host plant; B. Infection under stereomicroscope; C. Appresorium; D, E Conidia; F. Coniidiophore & foot cell. Scale bars: A = 5 cm; C–E = 15 μm; F = 40 μm.
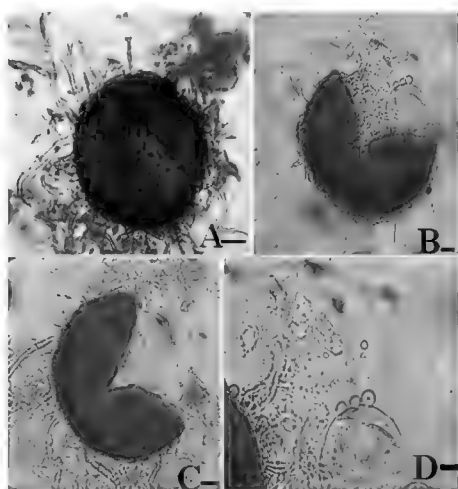
Fig. 2. *Erysiphe bistortae* (holotype, LAH 36943)A. Chasmothecium with appendages; B. Chasmothecium releasing ascospores; C. Ascus containing ascospores; D. Ascospores. Scale bars: A = 30 μm; B, C = 20 μm; D = 15 μm.

MYCELIUM amphigenous, also on stems, effused or in patches, persistent, 4-7 μm wide. HYPHAE hyaline, thin- and smooth-walled. CONIDIOPHORES erect from top of the mother cell, reaching up to a height of 107 μm. FOOT CELLS cylindrical, straight, 13-40 × 5-10 μm, followed by 1-2 shorter cells and single conidium. CONIDIA ovoid to cylindrical, 28-48 × 13-20 μm, germ tubes arising from an end, short to quite long, 0.25-3.5 times the conidial width. APPRESSORIA with 0-9 lobes, variable, 3-8 μm diam. CHASMOTHECIA scattered to gregarious, dark brown in color, globose to sub globose, 105-163(-165) μm diam. PERIDIUM CELLS inconspicuous, irregularly hexagonal, 8-17 μm diam. APPENDAGES numerous, variable in number, mycelioid, simple or frequently irregularly branched, often in a coral-like manner, densely crowded and interwoven mycelium, 0.5-1.4 times as long as the chasmothecial diam., 3-6(-7) μm wide, septate, often with only a few septa, thin-walled, smooth, pigmented when mature, yellow to brown throughout or paler towards the tip. ASCI (2-)3-5, broad ellipsoid, obovoid, saccate, 44-75 × (35-)30-54 μm, short stalked, rarely sessile, (2-)3-5-spored. ASCOSPORES ellipsoid to ovoid, 25-34 ×

12–17 μm, colorless.

ADDITIONAL SPECIMEN EXAMINED—**PAKISTAN, KHYBER PAKHTUNKHWA, Abbottabad**, Thandiani, 34.23°N 73.35°E, 2500 m alt., on *Bistorta amplexicaulis*, 13 Aug. 2020, N.S. Afshan and A.R. Niazi JP-07 (LAH36143; GenBank MZ048849).

Discussion

Two nr ITS DNA sequences of the local collections (JP-7 and LDH-01) were generated and BLAST searched at https://blast.ncbi.nlm.nih.gov/. These showed sequence similarity of 97.44% with *Erysiphe betae* (Vaňha) Weltzien (MN368297) and 98.40% with *E. betae* (KX574674). Closely related ingroup sequences were retrieved from GenBank and published literature (Takamatsu & al. 2015) along with an outgroup sequence *Erysiphe mori* (I. Miyake) U. Braun & S. Takam. (KY910120). All retrieved sequences, together with our new Pakistani sequences, were aligned through MAFFT (Multiple sequence alignment tool). The final data set contained 596 positions out of which 453 were conserved, 208 were variable and parsimony uninformative, and 51 were parsimony informative. The final data set was analyzed on MEGA 6.0 for assessing its correct position. The newly generated Pakistani sequences (JP-7 and LDH-01) clustered within the *E. betae/E. heraclei/E. malvae* complex lineage with a strong bootstrap support (FIG. 3). This complex consists of *E. betae* (KY399969, KX574674, KF268348, MN368297), *E. heraclei* DC. (KY661135, KY660878, LC010004, KF111807, KR048065, MT174194, MT174193, KP729443), and *E. malvae* V.P. Heluta (LC010041, LC010040). Other sequences closely related to the Pakistani collections on *Bistorta amplexicaulis* pertain to *E. polygoni* (LC328322, LC328323, MW169023, AF011307, KY661154) (FIG. 3).

The new Pakistani sequences showed 98.40 and 97.7% sequence similarity with *E. betae* (KX574674, KY399969, MN368297, KF268348), 98.90% with *E. heraclei* (KY661135, KY660878, LC010004, KF111807, KR048065, MT174194, MT174193, KP729443), and 98.81% with *E. malvae* (LC010041, LC010040) within the complex; and 97.92% sequence similarity with *E. polygoni* (LC328322, LC328323, MW169023, AF011307, KY661154).

The new Pakistani sequences showed two nucleotide differences from *E. heraclei* (KY661135, KY660878, LC010004), three from *E. heraclei* (KF111807, KR048065), four from *E. heraclei* (MT174194, MT174193), and five from *E. heraclei* (KP729443); two nucleotide differences from *E. betae* (KY399969, KX574674, KF268348), zero difference from *E. betae* (MN368297); two nucleotide differences from *E. malvae* (LC010041, LC010040); and nine nucleotide differences compared from *E. polygoni* (AF011307), eight from
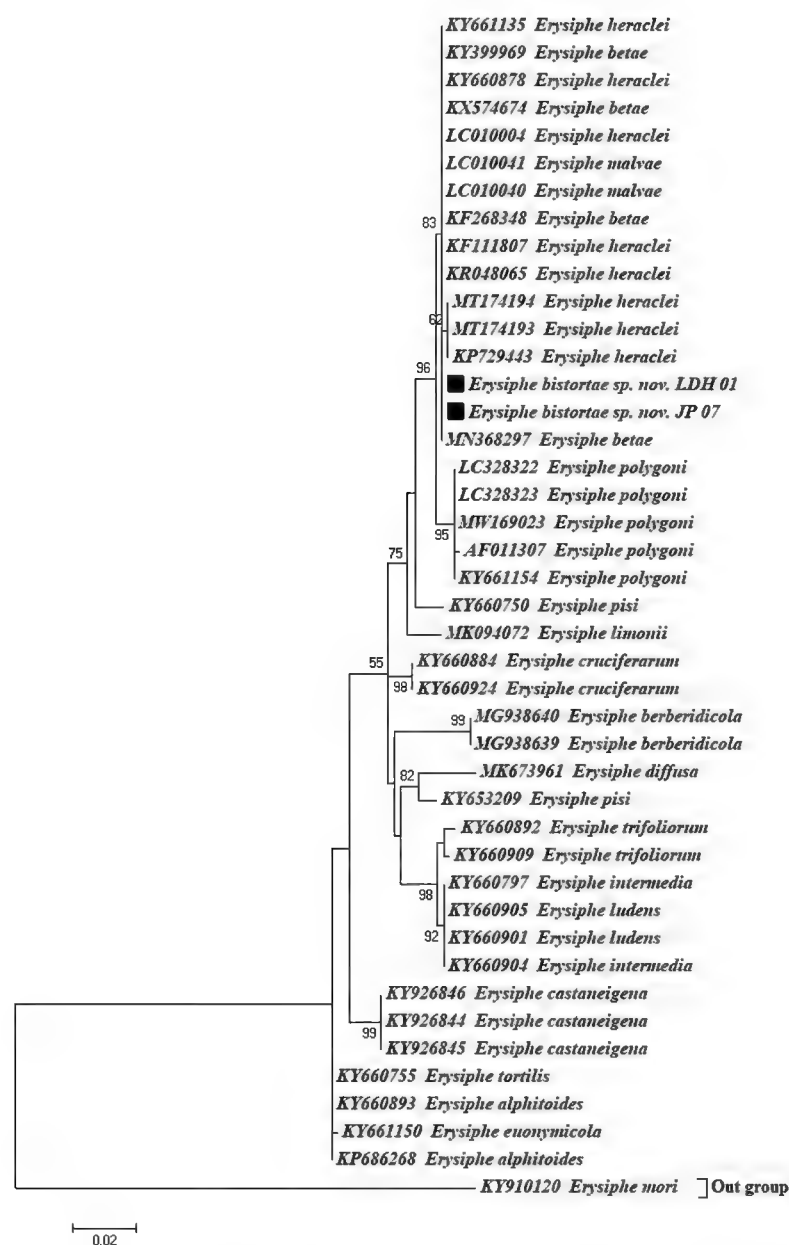
Fig. 3. Phylogenetic analysis of *Erysiphe bistortae* based on a Maximum likelihood analysis of the ITS region. The tree was rooted using *Erysiphe mori*. The ML bootstrap value is shown adjacent to each branch. The new sequence from Pakistan is marked with ▪.

E. polygoni (KY661154) and seven from *E. polygoni* (LC328322, LC328323, MW169023).

Erysiphe bistortae, the new powdery mildew species on *Bistorta amplexicaulis* in Pakistan, belongs morphologically as well as genetically to the *Erysiphe betae*/*E. heraclei*/*E. malvae* complex (cluster), with *E. polygoni* as sister group (Takamatsu & al. 2015). All species included in this complex are phylogenetically closely allied and morphologically characterized by having rather similar asexual morphs and chasmothecia with frequently irregularly branched appendages. At first glance, one would be inclined to assign the

Erysiphe on *Bistorta* (*Polygonaceae*) to *Erysiphe polygoni*, described from Europe on *Polygonum aviculare* L. as type host, a species pertaining to *Polygonum* s.str. *Bistorta* spp. are unknown as hosts of this species in Europe and in general (Braun & Cook 2012). The asexual morph of *E. polygoni* agrees well with collections on *Bistorta amplexicaulis*, and the *E. polygoni* chasmothecia are also close but smaller (85–140 µm diam.; Braun & Cook 2012). *Erysiphe polygoni* is biologically specialized, and the powdery mildew on *Polygonum aviculare* s.lat., the type host, is confined to that host (= f.sp. *polygoni-avicularis*; Hammarlund 1925, Blumer 1967), and sequences retrieved from *B. amplexicaulis* cluster within the *Erysiphe betae/E. heraclei/E. malvae* complex, whereas *E. polygoni* clusters separately. The *Erysiphe betae/E. heraclei/E. malvae* complex (cluster) consists of species that cannot be clearly distinguished solely by their ITS sequences (Takamatsu & al. 2015), a situation that is comparable with the *Erysiphe aquilegiae* DC. complex (Shin & al. 2019, Bradshaw & al. 2021). However, on account of biological and morphological differences within this complex, the Asian powdery mildew on *B. amplexicaulis* warrants description as a species of its own. *Erysiphe betae* differs morphologically in having smaller chasmothecia, 75–135 µm diam., and conidiophore foot-cells predominantly formed by a short foot-cell followed by a longer second cell and 1–2 shorter cells; and *Beta* powdery mildew is biologically specialized and confined to *Beta* spp. (*Amaranthaceae*; Drandarevski 1978). *Erysiphe heraclei* is confined to hosts of the *Apiaceae*, with several formae speciales (Hammarlund 1925, Blumer 1967, Dixon 1978), and this species is morphologically distinguished from *E. bistortae* by longer conidiophore foot-cells (20–70 µm), often with a second cell longer than the foot-cell (Braun & Cook 2012). *Erysiphe malvae* differs in having longer conidiophore foot-cells, 35–70 µm, and smaller chasmothecia, 100–140 µm diam. (Braun & Cook 2012).

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MYCOTAXON

Puccinia malvacearum and *P. xanthii* newly recorded from Pakistan

N.S. Afshan[*], M. Riaz, M.A. Ahmad, A.N. Khalid

Institute of Botany, University of the Punjab
    Quaid-e-Azam Campus-54590, Lahore, Pakistan

[*] Correspondence to: *najamulsehar.botany@pu.edu.pk, pakrust@gmail.com*

Abstract—During a survey of plant pathogenic fungi of Parachinar, Khyber Pakhtunkhwa, Pakistan, two plant species, *Malva neglecta* (*Malvaceae*) and *Xanthium strumarium* (*Asteraceae*), were found to be infected with rust fungi. After careful morphological observations, these rusts were identified as *Puccinia malvacearum* and *P. xanthii,* which are new records for Pakistan.

Key words— Cocklebur rust, Koh-e-Safaid, Kurram Agency, *Pucciniales*

Introduction

Parachinar is the capital city of Kurram District in Khyber Pakhtunkhwa, Pakistan. It is located at 33.9011°N 70.0860°E, at an altitude of 1726 m above sea level. Parachinar is bounded by high mountains on all sides and the major range is Koh-e-Safaid, which makes a natural boundary with Tora Bora mountain of Afghanistan and is covered with snow for most of the year. The name "Parachinar" comes from 'Para', one of the famous ancient tribes known as the 'Parikhel' and 'Chinar', the maple or pine trees which are found in abundance in the region. This area has some patches of oak and pine vegetation. Plant diversity, life style, and dispersion of plants are linked with the altitude and precipitation (Badshah & al. 2016). Due to its geographical location, little work has been done on floral diversity of this area, particularly fungal flora as we could not find any literature related to this aspect of natural vegetation. In order

to explore this floristically diverse but neglected area, visits were conducted in 2018 & 2021. During this exploration, two plant species, *Malva neglecta* and *Xanthium strumarium*, were found to be infected by rust fungi. After careful morphological analysis, these rusts were identified as *Puccinia malvacearum* and *P. xanthii*, which are presented here as new records for Pakistan. Previously, no rust fungus had been reported from this area.

Materials & methods

Plants infected with rust fungi were collected from Kurram District, Parachinar, Khyber Pakhtunkhwa province, Pakistan. Healthy plants with fruits and inflorescences were also collected for identification. Infected plants were photographed in the field and under the stereomicroscope. Host plants were identified by comparing with plants in the herbarium of Institute of Botany, University of the Punjab, Lahore (LAH).

Scratch mounts and free hand sections of infected portions were mounted in lactophenol. Partial permanent slides were made by cementing glass cover slips with nail lacquer; slides were examined under a biological microscope (LABOMED, Labo America, Inc. USA). Line drawings of different spore stages were made using a Camera Lucida (Ernst Leitz, Wetzlar, Germany). Dimensions of spores were taken using Scope Image 9.0(5X) with 40× objective. Forty spores were measured for each species. SEM micrographs were taken in the Centralized Resource Laboratory (CLR), Department of Physics, University of Peshawar, Pakistan. The specimens were deposited at the herbarium of the Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Taxonomy

Puccinia malvacearum Bertero ex Mont., Fl. Chil. 8: 43 (1852)   Fig. 1

Telia amphigenous, mostly hypophyllous, light brown to chestnut- brown, pustules scattered on surface of leaves and petioles. Teliospores 2-celled, 14–29 × 22–40(–52) μm; wall 2–4 μm thick at sides, light brown to hyaline or chestnut-brown, smooth, apex hyaline to light brown, 5–12 μm thick; pedicel long, hyaline, 5–15 × 48–163 μm.

Material examined: PAKISTAN, Khyber Pakhtunkhwa, Kurram District, Parachinar, 1726 m asl, on *Malva neglecta* Wallr. (*Malvaceae*), stage III, July 2018, Abdul Nasir Khalid NRPU#32 (LAH0718); Parachinar, Zeran, 1874 m asl, on *Malva neglecta*, stage III, 5 August 2021, Mohammad Aijaz Ahmad & Abdul Nasir Khalid NRPU#101 (LAH37120).
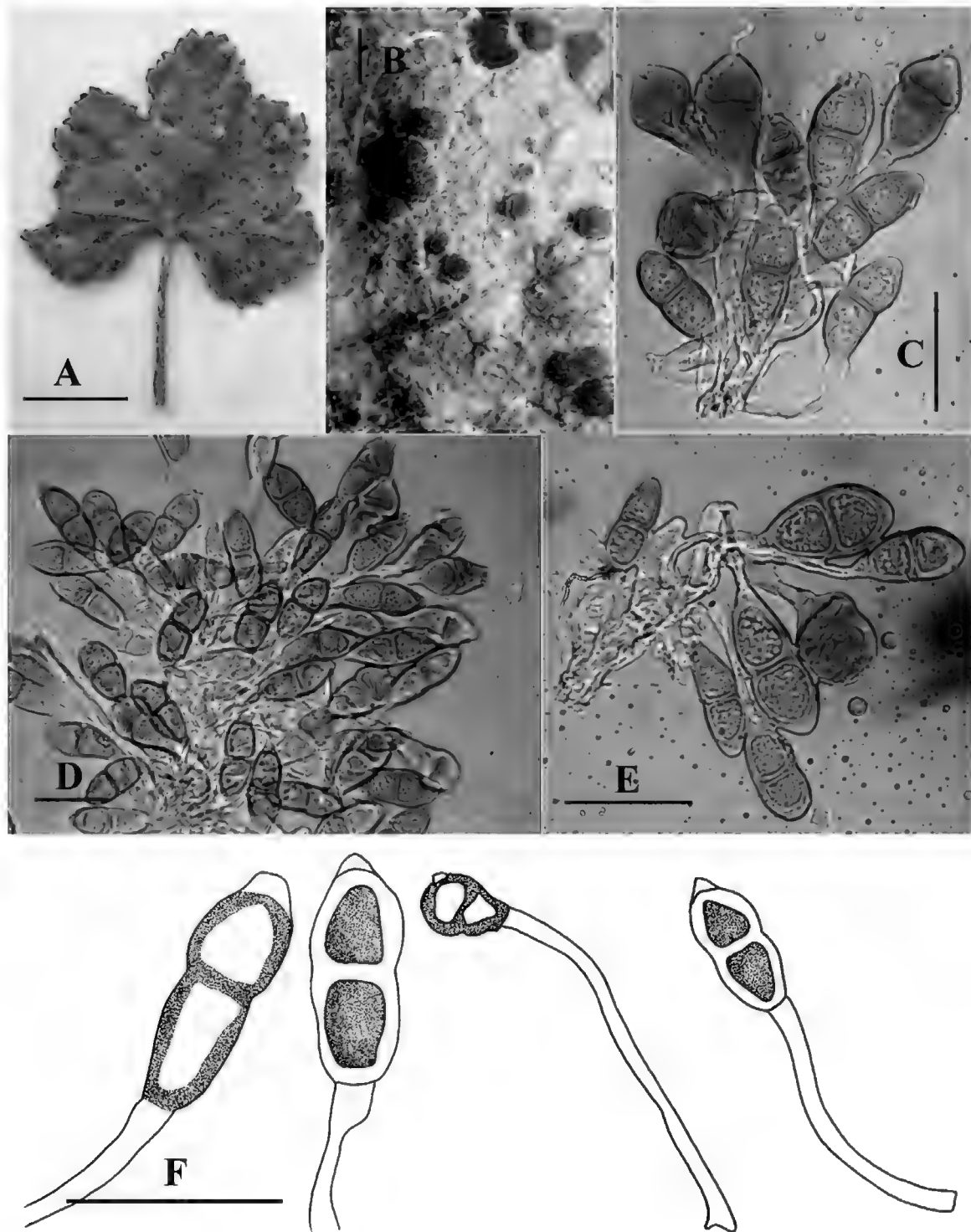
Fig. 1: *Puccinia malvacearum* (LAH0718). A. Infected leaf of *Malva neglecta*; B. Infection under stereomicroscope; C–F. Teliospores. Scale bars: A = 0.5 cm; B = 1 mm; C–F = 40 µm. (Drawing by Maria Riaz).

Comments—The size, color, and morphology of the teliospores fit the description of *Puccinia malvacearum* (Demers & al. 2015). *Puccinia malvacearum* was collected on *Malva neglecta* and represents a new record for Pakistan; and no other rust fungus has been reported on *Malva* spp. from Pakistan. *P. malvacearum* is cosmopolitan in distribution and has been reported

to infect several genera of the mallow family (*Malvaceae*) worldwide (Ahmad & al. 1997; Farr & Rossman 2022).

Puccinia xanthii Schwein., Schr. Naturf. Ges. Leipzig 1: 73 (1822)   Fig. 2
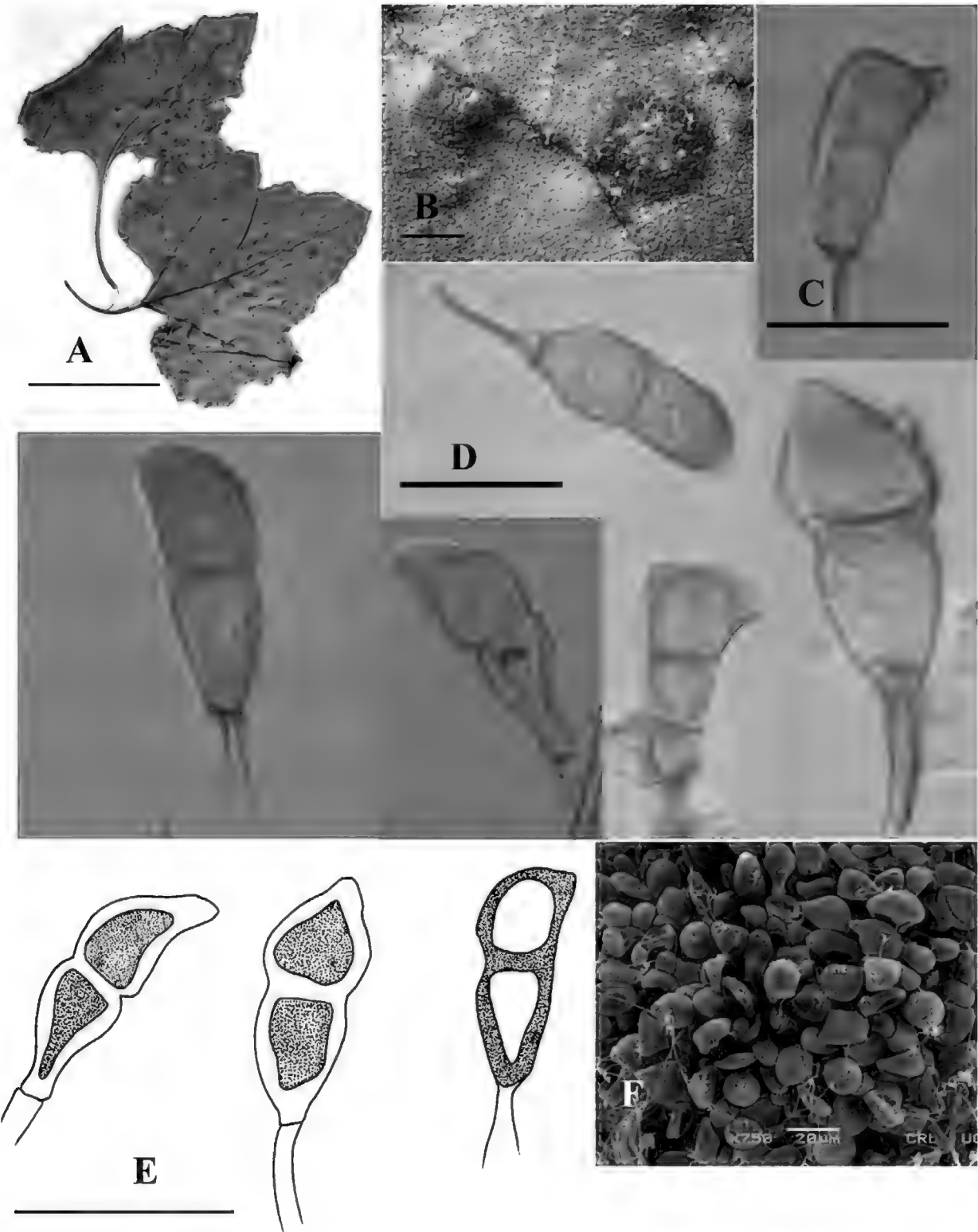
Fig. 2: *Puccinia xanthii* (LAH2307). A. Infected leaves of *Xanthium strumarium*; B. Infection under stereomicroscope; C–E. Teliospores; F. Teliospores under SEM. Scale bars: A = 5 cm; B = 1 mm; C–E = 40 μm. (Drawing by Maria Riaz).

Telia amphigenous, mostly hypophyllous, dark brown in circular patches on leaves. Teliospores 2-celled, clavate to ellipsoid, light brown to cinnamon-brown, 17–23 × 42–58 μm, wall 0.2–1.5 μm thick at sides; apex mostly conical, rarely rounded or truncate, light brown to cinnamon-brown, 5–10 μm thick; pedicel hyaline to light brown, 2–11 × 16–40 μm, mostly broken.

Material examined: **PAKISTAN, Khyber Pakhtunkhwa, Kurram District**, Parachinar, 1726 m asl, on *Xanthium strumarium* L. (*Asteraceae*), stage III, July 2018, Abdul Nasir Khalid NRPU#41 (LAH2307); Parachinar, Agra Valley, 1829 m asl, on *Xanthium strumarium*, stage III, 7 August 2021, Mohammad Aijaz Ahmad & Abdul Nasir Khalid NRPU#102 (LAH37121).

Comments—*Puccinia xanthii*, commonly known as cocklebur rust, is circumglobal on species of *Xanthium* L. and *Ambrosia* L. The size, color, and morphology of the teliospores fit the description of *Puccinia xanthii* (Dávid & al. 2003). No rust fungus has been reported on *Xanthium* spp. from Pakistan, and *P. xanthii* is a new record for the country; it has been reported on various species of *Xanthium* from different countries worldwide (Farr & Rossman 2022).

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Four species of *Rhizocarpon* subg. *Phaeothallus* in China

Yan-xue Bi[1], Yan-meng Zhang[1], Zun-tian Zhao[2*], Ling Hu[1*]

[1] *Institute of Environment and Ecology, Shandong Normal University,*
    Jinan, 250014, P. R. China
[2] *Key Laboratory of Plant Stress Research, College of Life Sciences,*
    Shandong Normal University, Jinan, 250014, P. R. China
Correspondence to: Hu_Ling_123@163.com, ztzhao@sdnu.edu.cn

Abstract—Three lichen species are reported from China for the first time: *Rhizocarpon cinereovirens*, *R. rittokense*, and *R. roridulum*. A revision of the Chinese material determined as *R. infernulum* f. *infernulum* shows that it belongs to *R. infernulum* f. *sylvaticum*. The morphology, secondary chemistry, ecology, and distribution ranges of the species are investigated and discussed. An identification key is given to the species of *Rhizocarpon* subg. *Phaeothallus* in China.

Key words—*Rhizocarpaceae, Rhizocarpales*, saxicolous, taxonomy

Introduction

Rhizocarpon (*Rhizocarpaceae*) was validated by De Candolle in 1805 (Kirk & al. 2008). It is widely distributed on rocks or occurs as parasite on other lichens throughout the world, especially in alpine and polar regions (Matwiejuk 2008). *Rhizocarpon* is characterized by having crustose thallus, usually distinct black prothallus, lecideine apothecia, richly branched and anastomosing paraphyses, *Rhizocarpon*-type and (1–)8-spored asci and hyaline to brown or olive-green, halonate, ellipsoid ascospores that can be transversely septate or submuriform to densely muriform (Feuerer & Timdal 2004, Fletcher & al. 2009, McCarthy & Elix 2014). Thomson (1967) divided the genus *Rhizocarpon* into taxa with a yellow thallus containing rhizocarpic acid (*R.* subg. *Rhizocarpon*) and taxa with

non-yellow (white, ashy, or brown) thallus lacking rhizocarpic acid (*R.* subg. *Phaeothallus*).

Rhizocarpon includes about 235 species worldwide (Lücking & al. 2016; Davydov & Yakovchenko 2017; Etayo 2017; Kalb & Aptroot 2017; Paukov & al. 2017; Kondratyuk & al. 2018; Fryday 2019; Elix & McCarthy 2019; McCarthy & al. 2020; Spribille & al. 2020), of which 47 have been reported from China (Wei 1991, 2020; Abbas & Wu 1998; Aptroot 2002; Aptroot & Sparrius 2003; Sérusiaux & al. 2003; Golubkov & Maywiejuk 2009; Li & al. 2013; Zhao & al. 2013; Mahire & al. 2015; Wang & al. 2015a,b,c, 2016; Gulina & Anwar 2019; Hu & al. 2020).

During our study of *Rhizocarpon* in China, three additional species have been found: *R. cinereovirens, R. rittokense,* and *R. roridulum*; a revision of the Chinese material determined as *R. infernulum* f. *infernulum* showed that it belongs to *R. infernulum* f. *sylvaticum.* An identification key is provided for the 31 species of *Rhizocarpon* subg. *Phaeothallus* known from China.

Materials & methods

The examined specimens are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU) and the Sectio Lichenum of Herbarium Mycologici Academiae Sinicae, Chinese Academy of Sciences, Beijing, China (HMAS-L). Macromorphological characters were examined under a stereomicroscope (COIC XTL7045B2) and micromorphological characters under a polarizing microscope (Olympus CX41). For identification, the thallus and medulla were tested with K (a 10% aqueous solution of potassium hydroxide), C (a solution of aqueous sodium hypochlorite), I (Lugol's iodine), N (a 50% aqueous solution of nitric acid), and P (a saturated solution of p-phenylenediamine in 95% ethyl alcohol). Lichen substances were identified using standardized thin-layer chromatography techniques (TLC) with solvent system C (Orange & al. 2010). Photographs were taken with an Olympus SZX16 and a BX61 microscope with a DP72 lens.

Taxonomy

Rhizocarpon cinereovirens (Müll. Arg.) Vain., Acta Soc. Fauna Fl. Fenn. 53(1): 336 (1922)   Fig. 1

THALLUS crustose, cream-colored to pale brown, areolate to bullate, 2–5 cm diam.; areoles rounded to angular, flat to convex, smooth, continuous, 0.2–0.4 mm diam. PROTHALLUS absent. MEDULLA I–. APOTHECIA lecideine,

Fɪɢ. 1. *Rhizocarpon cinereovirens* (HMAS-L–Gao114944). A. Thallus; B. Apothecia; C. Apothecium section; D. Crystals in exciple and epihymenium; E. Ascus; F. Ascospores. Scale bars: A = 1 mm; B = 100 μm; C, D = 20 μm; E, F = 10 μm.

black, sessile to somewhat immersed, 0.3–0.6 mm diam., rounded to angular, flat to weakly convex; margin thin, epruinose. Exciple dark brown, K–, with crystals dissolving in K; epihymenium dark green, K+ green, with crystals dissolving in K; hymenium hyaline; paraphyses branched and anastomosing, scarcely swelling at apex, with a green-blue cap; hypothecium dark brown. Asci 8-spored. Ascospores hyaline, 1-septate, 15–17 × 6.5–8.5 μm, halonate. Conidiomata not seen.

CHEMISTRY—Medulla K+ yellow turning red, C–, P+ yellow. Norstictic acid and stictic acid detected by TLC.

SPECIMEN EXAMINED: **CHINA. JILIN, Shulan City**, Qingsong forestry station, alt. 600 m, on siliceous rock, 8 Oct. 1984, Xiangqun Gao 114944 (HMAS-L).

DISTRIBUTION—Norway, Denmark, Great Britain, Finland, Sweden, Switzerland, Canada, USA (Thomson 1967, Fryday 2002). New to China and to Asia.

COMMENTS—Our specimen conforms to previously published descriptions (Fryday 2002, Fletcher & al. 2009). *Rhizocarpon cinereovirens* is characterized by the cream-colored to pale brown thallus, dark green epihymenium with crystals, hyaline, 1-septate ascospores and by containing norstictic and stictic acid. *Rhizocarpon cinereovirens* is somewhat similar to *R. discoense* Lynge by paraphyses with greenish cap at apex and hyaline 1-septate ascospores, but *R. discoense* has a well-developed exciple with a violet pigmentation (K+ violet), a thallus with grey bullate areoles, and wider ascospores 16–17 × 9–10 μm (Fryday 2002, Fletcher & al. 2009). *Rhizocarpon cinereovirens* is also similar to *R. infernulum* (Nyl.) Lynge f. *infernulum*, which has a more well-developed exciple with a hyaline interior, and less richly branched and anastomosing paraphyses with more sharply defined caps that are brown rather than green. In additon, the lichen substances of *R. infernulum* f. *infernulum* are ± stictic acid (Fryday 2002, Fletcher & al. 2009).

Rhizocarpon rittokense (Hellb.) Th. Fr., Lichenogr. Scand. 2: 615 (1874)   FIG. 2

THALLUS crustose, brown, areolate to subsquamulose, 3–4 cm diam.; areoles peltate, concave, shining, smooth, scattered, often with pruinose margin, 0.2–1 mm diam. PROTHALLUS present, black. MEDULLA I–. APOTHECIA lecideine, black, immersed, 0.2–0.5 mm diam., rounded, flat; margin thin, epruinose. EXCIPLE dark brown, K+ violet, with crystals dissolved in K; epihymenium brown, K+ violet, with crystals dissolved in K; hymenium hyaline; paraphyses branched and anastomosing, with a sharply delimited, brown-pigmented cap; hypothecium dark brown. ASCI 8-spored. ASCOSPORES brown-green to dark brown, 1-septate, 18–23 × 11.5–15 μm, halonate. CONIDIOMATA not seen.

CHEMISTRY—Medulla K–, C–, P–. Barbatic acid detected by TLC.

SPECIMENS EXAMINED: **CHINA. SICHUAN, Luding Co.**, Moxi Town, Yajiagen, alt. 4000 m, 29.9100°N 102.0038°E, on siliceous rock, 13 Oct. 2015, Weicheng Wang, Xiangxiang Zhao, Feixiang Shi, Zuntian Zhao 20150587 (SDNU). **YUNNAN, Lijiang City**, Mt. Laojun, alt. 3850 m, on siliceous rock, 25 Aug. 2015, Weicheng Wang 20150513 (SDNU).
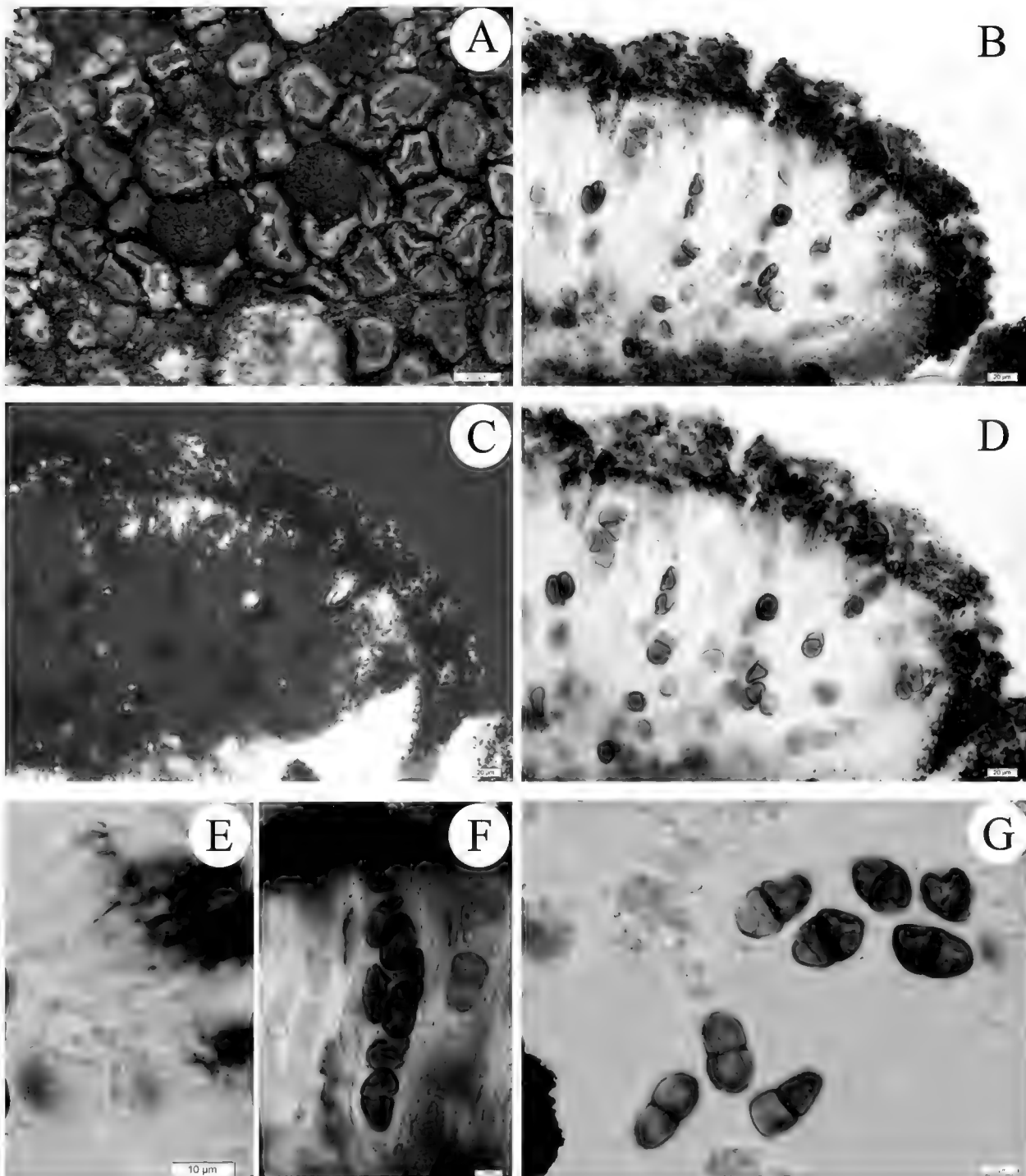
FIG. 2. *Rhizocarpon rittokense* (SDNU–Wang20150587). A. Thallus and apothecia; B. Apothecium section; C. Crystals in exciple and epihymenium; D. K reaction; E. Paraphyses; F. Ascus; G. Ascospores. Scale bars: A = 200 μm; B–D = 20 μm; E–G = 10 μm.

DISTRIBUTION—Sweden, Denmark, Norway, Iceland, Canada, USA, Russia, Norway (Lynge 1932, Thomson 1967, Timdal & Holtan-Hartwig 1988). New to China.

COMMENTS—Our specimens conform to previously published descriptions, except for the presence of the brown-green ascospores (Thomson 1967). *Rhizocarpon rittokense* is characterized by K+ violet exciple with crystals, K+

violet epihymenium with crystals and the subsquamulose thallus. *Rhizocarpon rittokense* is similar in habitat and 1-septate ascospores to *R. groenlandicum* Lynge, which differs by its convex apothecia with a bluish hymenium and its smaller ascospores (17–21 × 10–11 µm; Lynge 1932). *Rhizocarpon rittokense* is also similar to *R. cinereonigrum* Vain. in K+ violet epihymenium and exciple with crystals, but *R. cinereonigrum* differs from *R. rittokense* by K– exciple and larger ascospores (28–38 × 12–18 µm; Hu & al. 2020).

Rhizocarpon roridulum (Th. Fr.) Malme, Svensk Bot. Tidskr. 8(3): 279, 291 (1914)

FIG. 3

THALLUS crustose, gray to grayish brown, rimose to areolate, 1.5–2 cm diam.; areoles angular, flat, matt, scattered, 0.2–0.5 mm diam. PROTHALLUS absent. MEDULLA I–. APOTHECIA lecideine, black, 0.3–0.5 mm diam., somewhat immersed, rounded to angular, weakly concave; margin thick, epruinose. EXCIPLE dark brown, K+ violet, without crystals; epihymenium brown, K+ violet, without crystals; hymenium hyaline; paraphyses branched and anastomosing, scarcely swelling at apex, without strongly delimited pigmented cap; hypothecium dark brown. ASCI 8-spored. ASCOSPORES hyaline, submuriform, with 5–9 cells in optical section, 23–30 × 10.5–15 µm, halonate. CONIDIOMATA not seen.

CHEMISTRY—Medulla K–, C–, P–. No substance detected by TLC.

SPECIMEN EXAMINED: CHINA. SICHUAN, Litang Co., Mt. Qiazila, alt. 4250 m, on siliceous rock, 16 Oct. 2015, Zuntian Zhao, Feixiang Shi, Xiangxiang Zhao, Weicheng Wang 20150697 (SDNU).

DISTRIBUTION—Finland, Sweden, Norway (Fries 1874, Ihlen 2004). New to China and to Asia.

COMMENTS—Our specimen conforms to the description of Fries (1874). *Rhizocarpon roridulum* is characterized by the brown to grey-brown thallus, pruinose apothecia (but sometimes the pruina is missing), K+ violet epihymenium, and by not containing lichen substances (Ihlen 2004, Hu & al. 2020). *Rhizocarpon roridulum* is similar to *R. distinctum* Th. Fr. in the dark brown exciple and brown epihymenium, but *R. distinctum* usually has an I+ blue medulla, brown ascospores and contains stictic acid (Fryday 2000). *Rhizocarpon roridulum* is also similar in the K+ violet exciple to *R. geminatum* Körb., which can be distinguished by the 2-spored asci and larger ascospores (36–50 × 17–25 µm; McCarthy & Elix 2014). Additionally, *Rhizocarpon roridulum* is similar to *R. amphibium* (Fr.) Körb. in the predominantly reddish brown epihymenium
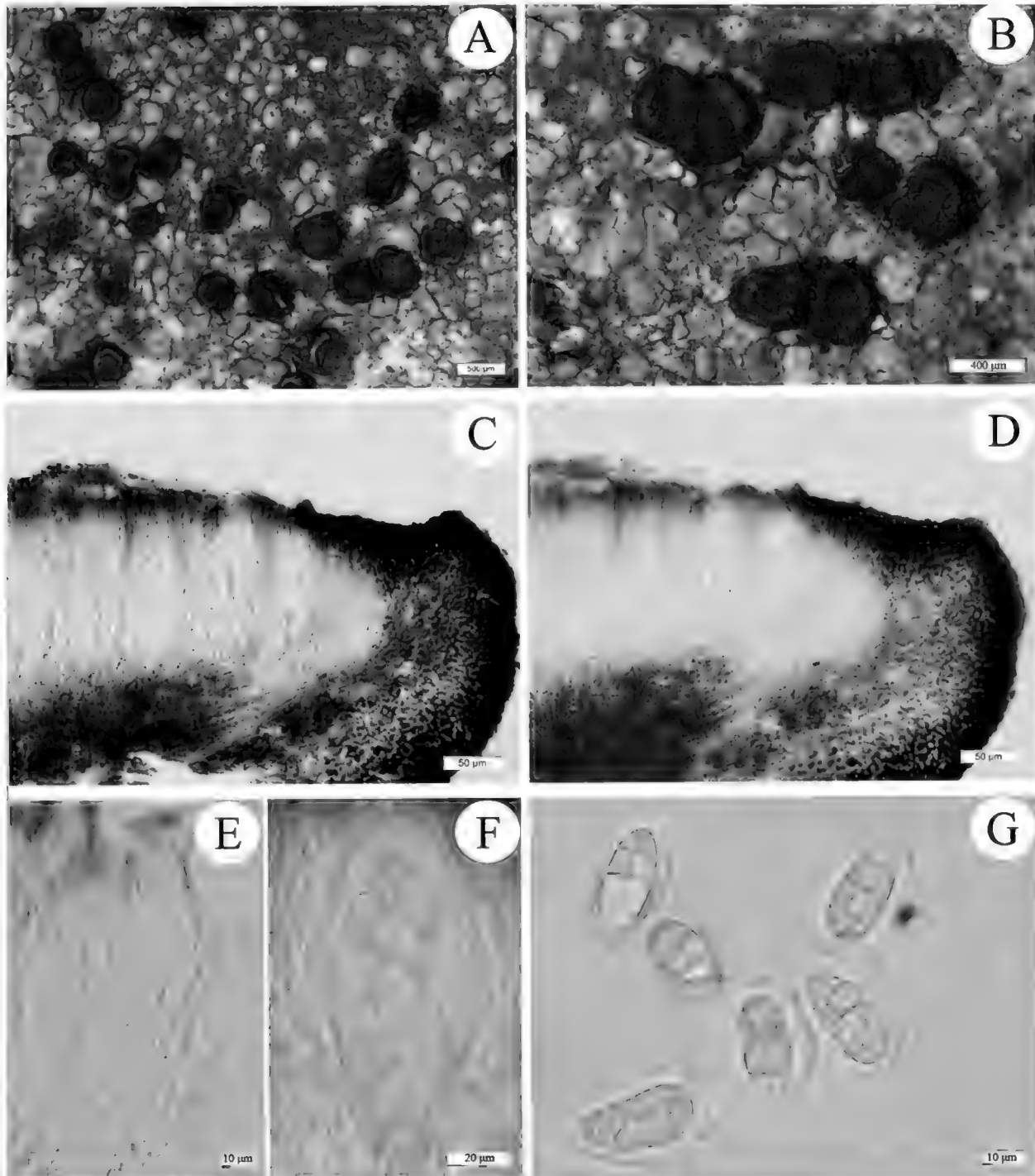
Fig. 3. *Rhizocarpon roridulum* (SDNU–Zhao20150697). A. Thallus; B. Apothecia; C. Apothecium section; D. K reaction; E. Paraphyses; F. Ascus; G. Ascospores. Scale bars: A = 500 μm; B = 400 μm; C, D = 50 μm; E, G = 10 μm; F = 20 μm.

with K+ purple reaction, but *R. amphibium* is characterized by the apothecia never with a white pruina and the exciple in section frequently with a green pigment in the outer and upper part (Ihlen 2004).

Revision

Rhizocarpon infernulum* f. *sylvaticum Fryday, Lichenologist 34(6): 468 (2002)

THALLUS crustose, brown, rimose to areolate, areoles rounded to angular, flat to weakly convex, matt, contiguous, 0.5 mm diam. PROTHALLUS absent. MEDULLA I–. APOTHECIA lecideine, black, 1 mm diam., sessile to somewhat immersed, rounded to angular, flat to weakly convex; margin thin, persistent, epruinose. EXCIPLE dark brown at the rim, inner part paler brown, K–, without crystals; epihymenium macrocarpa-green, K–, N+ red, without crystals; hymenium hyaline; paraphyses branched and anastomosing, with a sharply delimited, brown-pigmented cap; hypothecium dark brown. ASCI 8-spored. ASCOSPORES hyaline, 1-septate, (16–)17.5–20(–24) × 8–10 µm, halonate. CONIDIOMATA not seen.

CHEMISTRY—Medulla K–, C–, P–. No substance detected by TLC.

SPECIMENS EXAMINED: **CHINA. GUIZHOU, Leishan county**, Mt. Leigong, alt. 1800 m, on siliceous rock, 1 Apr. 2011, Yuliang Cheng 20112591A (SDNU); alt. 2100 m, on rock, 1 Apr. 2011, Xingran Kou 20111813 (SDNU); alt. 2700 m, on siliceous rock, 9 Nov. 2009, Lulu Zhang 20103078 (SDNU).

DISTRIBUTION—Great Britain, Ireland, Sweden, Canada, USA, China (Fryday 2002, Fletcher & al. 2009, Zhao & al. 2013).

COMMENTS—*Rhizocarpon infernulum* f. *sylvaticum* is characterized by the slightly larger spores and a smooth, cracked thallus, compared to the more areolate thallus and smaller spores (15–18 × 7–8.5 µm) of *R. infernulum* f. *infernulum*. Another difference between these two forms is the pigment of epihymenium: Fryday (2002) included the specimens with aeruginose (macrocarpa-green; K–, N+ red) to blue-black epihymenium in f. *infernulum*, and the specimens with invariably hyaline epihymenium in f. *sylvaticum*.

On the contrary, Möller (2021) found that the degree of epihymenium pigments varied within *R. infernulum* f. *sylvaticum*, some specimens had a very thin green epihymenium, and others had a very thick bright green epihymenium. During his investigation of the *R. hochstetteri* group by molecular methods, he found that specimens from clade A2 which he regarded as f. *sylvaticum* form a highly supported clade together with a specimen (11041) that Fryday himself determined as f. *sylvaticum*, and these specimens fit his observations of the type specimen. Moreover, the clade A2 (including f. *sylvaticum*) and A3 (including f. *infernulum*) were well supported and separated. He suggested (Möller 2021)

that f. *sylvatica* should be raised to species rank, but did not publish a formal proposal. Here, we retain the name *R. infernulum* f. *sylvaticum*.

Rhizocarpon infernulum f. *infernulum* was first reported from China by Zhao & al. (2013). Recently we re-examined those specimens (SDNU-20103078, 20111813, 20112591A) and we found that the spores measured (16–)17.5–20(–24) × 8–10 μm, much larger than the 15–18 × 7–8.5 μm published by Zhao & al. (2013). The specimen also had a thin, smooth, brown, cracked thallus, sessile apothecia with a persistent proper margin, so we reidentify the specimen as *R. infernulum* f. *sylvaticum*.

Key to the species of Rhizocarpon subg. Phaeothallus in China

1. Ascospores 1-septate 2
1. Ascospores 3-septate to muriform 13
2. Ascospores remaining hyaline or only finally darkening 3
2. Ascospores soon becoming dark green or brown 7
3. Thallus dark brown, medulla I+ blue .... *R. polycarpum*
3. Thallus ashy, gray, gray-brown or brown, medulla I– 4
4. Epihymenium and exciple containing crystals dissolved in K .... **R. cinereovirens**
4. Epihymenium and exciple not containing crystals 5
5. Thallus containing stictic acid; ascospores 22–27 × 9–12 μm .... *R. hochstetteri*
5. Thallus containing no lichen substances 6
6. Exciple K+ violet; ascospores 14–17 × 6–9 μm .... *R. expallescens*
6. Exciple K–, ascospores 17.5–20 × 8–11 μm .... **R. infernulum** f. **sylvaticum**
7. Medulla I+ blue, ascospores usually <20 μm long 8
7. Medulla I–, ascospores usually >20 μm long 9
8. Epihymenium brown-black; hymenium hyaline to pale brown, ascospores olive-green or brown, 12.5–16 × 6.5 μm .... *R. simillimum*
8. Epihymenium black; hymenium hyaline below, upper part pale red, ascospores brown, 12.5–17.5 × 6–7 μm .... *R. umense*
9. Epihymenium olive-brown to green-black, K–; containing norstictic acid or stictic acid, ascospores blue-green, 18–26 × 8–13 μm .... *R. copelandii*
9. Epihymenium red-brown to black brown, K+ violet 10
10. Exciple with crystals dissolved in K 11
10. Exciple without crystals 12
11. Exciple K-; ascospores 28–38 × 12–18 μm .... *R. cinereonigrum*
11. Exciple K+ violet; ascospores 18–23 × 11.5–15 μm .... **R. rittokense**
12. Thallus pale gray-brown to brown, containing no lichen substances; ascospores olive-brown to red-brown or brown, 25–35 × 12–15 μm .... *R. badioatrum*
12. Thallus gray to dark brown, containing gyrophoric acid; ascospores olive-brown, 35–40 × 10–15 μm .... *R. sinense*
13. Asci 1- or 2-spored 14
13. Asci 8-spored 17

29. Medulla K+ yellow or red 30
30. Medulla K+ yellow, containing stictic acid .... *R. petraeum*
30. Medulla K+ red, containing norstictic acid .... *R. rubescens*

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Usnea jingdongensis sp. nov. from Southwest China

Yu-xin Chen[1], Li-bao Wang[2], Yu-huan Xie[1], Shou-yu Guo[3*], Liu-fu Han[1*]

[1] *College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, P. R. China*

[2] *College of Home Economics, Hebei Normal University, Shijiazhuang 050024, P. R. China*

[3] *State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P. R. China*

[*] Correspondence to: *guosy@im.ac.cn, hanliufu@hebtu.edu.cn*

Abstract—*Usnea jingdongensis* from the Ailaoshan Mountain of Southwest China, is described as a new species of *Parmeliaceae*. It is characterized by the uninflated branches, fistulose axis with pale brown to dark brown loose hyphae, and the absence of pseudocyphellae and soralia. The phylogenetic analysis of the nrDNA ITS sequence data supported the recognition of the species. A key to the eumitrioid *Usnea* species in China is also provided.

Key words—Asia, evolutionary tree, *Lecanorales*, protocetraric acid, taxonomy

Introduction

Usnea Dill. ex Adans. is a hyperdiverse lichen genus, with more than 350 species (Clerc 1998; Kirk & al. 2008; Lücking & al. 2017) distributed from polar zones to tropical areas. It forms a strongly supported monophyletic lineage (Crespo & al. 2007) in the family *Parmeliaceae* (*Lecanorales*, *Lecanoromycetes*, *Ascomycota*). Traditional diagnostic characters of the genus include a fruticose thallus, branches with a cartilaginous central axis, and the presence of usnic acid (Clerc 1998; Ohmura 2001). In taxonomic studies, these characters were proved to be feasible for genus delimitation (Clerc 1998; Ohmura 2001). However, at the species level, the plasticity of morphological characteristics to environmental changes poses a great challenge for the delimitation of species (Truong & Clerc 2013). One of the DNA barcoding markers, the nuclear ribosomal internal transcribed spacer (nrDNA ITS), had been proposed for

fungi, including lichenized fungi (Schoch & al. 2012). A series of phylogenetic studies supported the delimitation of species in *Usnea* described from morphological and chemical features (Ohmura 2008; Liu & Guo 2009; Saag & al. 2011; Gerlach & al. 2019; Temu & al. 2019; Lücking & al. 2020). Eumitrioid *Usnea* (= *Eumitria*), comprising the species with a tubular central axis ± filled with loose hyphae throughout the branches of the thallus, has consistently been recovered as a monophyletic group in all phylogenetic studies (Articus 2004; Temu & al. 2019; Lücking & al. 2020).

Eumitria was described as a genus by Stirton (1882), but has usually been considered to be a subgenus of *Usnea* (Motyka 1936; Ohmura 2001, 2002). Based on the phylogenetic analysis of limited molecular data, Articus (2004) resurrected it at generic level, which was also accepted by Divakar & al. (2017), Kraichak & al. (2017), and Lücking & al. (2020).

Since only a small proportion of species (3/24) with a hollow, often fistulate central axis were included in the phylogeny, and these limited phenotypic features are not always easy to discern and are not completely consistent with the recognized genera, we defend the recognition of a single genus *Usnea* that is morphologically well characterized (Ohmura & Kanda 2004; Wirtz & al. 2006; Thell & al. 2018; Temu & al. 2019). Here, we prefer to use the term 'eumitrioid' for the *Usnea* species with a tubular central axis throughout the whole thallus (Truong & Clerc 2013).

Eumitrioid *Usnea* has been studied in Africa (Motyka 1936; Dodge 1956; Swinscow & Krog 1974, 1986; Krog 1994; Nadel 2016; Temu & al. 2019), Australia (Rogers & Stevens 1988; Stevens 1999), East Asia (Zhao & al. 1982; Ohmura 2001, 2012), and South America including the Galapagos (Truong & Clerc 2013).

Wei (1991) enumerated about 80 species of *Usnea* reported in the literature for lichen collections from China before 1989. Since then, a series of studies on the diversity and taxonomy of *Usnea* from China have been carried out (Aptroot & al. 1999, 2002; Ohmura 2001, 2012; Seaward & Aptroot 2005; Zhang & al. 2006; Ohmura & al. 2010; Han & al. 2020). Approximately 90 species of *Usnea* have now been reported from China, among which seven species are eumitrioid (Zhao & al. 1982; Ohmura 2001, 2012; Han & al. 2020; Wei 2020).

During the study of *Usnea* in China, we discovered a new eumitrioid species based on morphological characteristics and nrDNA ITS sequence data. It is here therefore formally described and its morphological and structural characteristics are compared with those of closely related species.

Materials & Methods

Specimens, morphology and chemistry

All specimens examined were collected from Mt. Ailaoshan, Yunnan Province, Southwest China and were deposited in the Herbarium Mycologicum Academiae Sinicae-Lichenes, Beijing, China (HMAS-L) and the Herbarium of Hebei Normal University, Shijiazhuang, China (HBNU). Morphological characters of the specimens were examined using stereomicroscopes (Motic SMZ-140 and Zeiss-SteREO Discovery-V12) and compound microscope (Leica DM500). The micromorphological structure of the branches was analyzed according to Ohmura (2001). The sizes of cortex, medulla, and central axis were measured in longitudinal sections of branches at 40× magnification. The relative thickness of cortex/medulla/axis of the total branch diameter (CMA) and the ratio of axis/medulla (A/M) of all the studied specimens were calculated according to Clerc (1984, 1987) and were ascribed to the categories defined by Clerc (2011). Percentages of the tubular part of the axis (TBA) were calculated according to Truong & Clerc (2013). The apothecia were cut by hand with a razor and mounted in water. The slices were stained with 0.2% toluidine blue for about 15 min. The asci and ascospores of apothecia were observed and photographed at 400× magnification with Leica DM500 microscope. Thin layer chromatography (TLC) was performed on all specimens examined using solvent systems C and G (Orange & al. 2010).

DNA extraction, PCR amplification and sequencing

The samples of branch tips were cut for DNA extraction from the specimens examined. DNA was extracted using the DNAsecure Plant DNA Kit (DN-14, Aidlab Biotechnologies Co., Ltd) following the manufacturer's protocol. The nrDNA ITS region (ITS1+5.8S+ITS2) was amplified according to Han & al. (2020). The primers ITS1F-forward (5'–CTT GGT CAT TTA GAG GAA GTAA–3', 22 nt) (Gardes & Bruns 1993) and ITS4-reverse (5'–TCC TCC GCT TAT TGA TAT GC–3', 20 nt) (White & al. 1990) were used for the Polymerase Chain Reaction (PCR). The amplification reaction was performed in a 25 μL volume containing 0.75 units of TransStart Taq Polymerase (Tiangen, China), 2.5 μL of buffer, 0.5 μL of a 5 μM solution of the primers, 2 μL of 2.5 mM for each dNTP solution, and 1 μL of genomic DNA. PCR cycling was performed with the following protocol: initial denaturation for 3 min at 95°C; 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min; with final elongation at 72°C for 10 min. PCR products were screened on 1% agarose gels stained with ethidium bromide and the sequences were generated by Genewiz Inc. (Suzhou, Jiangsu, China).

Phylogenetic analysis and sequence comparing

Seven newly obtained sequences (two from the holotype; one each from five other specimens) were submitted to GenBank (Table 1).

The Blastn (NCBI) searches were carried out by using the entire nrDNA ITS (ITS1+5.8S+ITS2) sequences and the searches were limited to records that excluded uncultured/environmental sample sequences, and the results were filtered to match

TABLE 1. Specimens and sequences of *Usnea* and *Eumitria* spp. used in the ITS phylogenetic analyses. Newly produced sequences are in bold, with the holotype annotated with [T].

SPECIES	COUNTRY	VOUCHER	GENBANK #	REFERENCE
U. baileyi	Japan	Ohmura 4488A	AB051050	Ohmura 2002
	USA	Ohmura 4516	AB051051	Ohmura 2002
	China	TNM L00004731	FJ494924	—
	Tanzania	Temu SGT156	MN080245	Temu & al. 2019
	Tanzania	Temu SGT119	MN080248	Temu & al. 2019
	Tanzania	Temu SGT112	MN080249	Temu & al. 2019
	Tanzania	Temu SGT110	MN080250	Temu & al. 2019
	Tanzania	Temu SGT63	MN080251	Temu & al. 2019
E. cf. *baileyi*	São Tomé & Principe	MN0417	MW267140	Nadel 2016
	São Tomé & Principe	MN0600a	MW267144	Nadel 2016
	São Tomé & Principe	MN0179	MW267145	Nadel 2016
E. firmula	São Tomé & Principe	MN0117	MW267133	Nadel 2016
	São Tomé & Principe	MN0581	MW267134	Nadel 2016
	São Tomé & Principe	MN0550b	MW267135	Nadel 2016
	São Tomé & Principe	MN0550a	MW267136	Nadel 2016
	São Tomé & Principe	MN0084	MW267137	Nadel 2016
U. glabrescens	Estonia	glabrescens_02	JN086304	Saag & al. 2011
	UK	glabrescens_15	JN086307	Saag & al. 2011
	Finland	glabrescens_16	JN086308	Saag & al. 2011
U. jingdongensis	**China**	**2018120543 [T]**	**MN080697**	**This study**
	China	**2018120543 [T]**	**MT261829**	**This study**
	China	**2018120562b**	**MN080698**	**This study**
	China	**2018120582**	**MN080700**	**This study**
	China	**2018120532**	**MN080702**	**This study**
	China	**2018120566**	**MN080704**	**This study**
	China	**2018082322a**	**MN080706**	**This study**
U. pectinata	China	TNM L00004729	FJ494946	—
	Tanzania	Temu SGT109	MN080236	Temu & al. 2019
	Tanzania	Temu SGT115	MN080237	Temu & al. 2019
E. pectinata	Japan	Ohmura 2989	AB051655	Ohmura 2002
	Indonesia	Ohmura 4373	AB051656	Ohmura 2002
E. cf. *pectinata*	São Tomé & Principe	MN0068a	MW267147	Nadel 2016
	São Tomé & Principe	MN0125	MW267148	Nadel 2016
	São Tomé & Principe	MN0597	MW267149	Nadel 2016

Species	Country	Voucher	GenBank #	Reference
	São Tomé & Principe	MN0060	MW267150	Nadel 2016
	São Tomé & Principe	MN0070b	MW267151	Nadel 2016
	São Tomé & Principe	MN0583	MW267162	Nadel 2016
	São Tomé & Principe	MN0063	MW267163	Nadel 2016
	São Tomé & Principe	MN0556	MW267164	Nadel 2016
	São Tomé & Principe	MN0567	MW267165	Nadel 2016
	São Tomé & Principe	MN0585	MW267166	Nadel 2016
E. aff. *pectinata*	São Tomé & Principe	MN0481	MW267153	Nadel 2016
	São Tomé & Principe	MN0065	MW267156	Nadel 2016
	São Tomé & Principe	MN0163	MW267157	Nadel 2016
U. wasmuthii	Norway	O-L-198061	MK812232	Marthinsen & al. 2019
	Colombia	41018b	MW241067	Moncada & al. 2020
Outgroup:				
U. diffracta	Japan	Ohmura 1124	AB051057	Ohmura 2002
	China	TNM L00004750	FJ494931	—
U. longissima	Japan	Ohmura 3664	AB051645	Ohmura 2002
	Japan	Ohmura 3816B	AB051647	Ohmura 2002
	China	TNM L00004685	FJ494936	—
U. trichodeoides	Japan	Ohmura 3809	AB051670	Ohmura 2002
	China	TNM L00004677	FJ494954	—

records with query coverage more than 95%, and percent identity between 88% and 100%. The representative taxa were selected mainly according to the Blastn search results in GenBank, morphological characters, and some references (Ohmura 2001, 2002; Articus 2004; Ohmura & Kanda 2004; Wirtz & al. 2006; Truong & Clerc 2013; Thell & al. 2018; Temu & al. 2019; Lücking & al. 2020).

The entire ITS sequences of seven samples of our examined specimens and the 46 representatives selected were aligned both by ClustalW and Muscle implemented in MEGA 6 (Tamura & al. 2013), then adjusted manually. The final data matrix can be obtained from the corresponding authors and was deposited in TreeBase (S29861).

Two model selection strategies Akaike (corrected) and Bayesian information criteria (AICc and BIC) implemented in MEGA 6 were used to determine the substitution models. The models K2+G with the lowest BIC score and GTR+G+I with the lowest AICc value were chosen to infer the phylogenetic trees by using the Maximum Likelihood (ML) in MEGA 6. For ML analysis, initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The analyses performed a non-parametric bootstrap analysis (1000 replicates) to assess branch support.

Result

nrDNA ITS sequences and the phylogenetic analysis

The ITS region was successfully sequenced for six specimens (two sequences for the holotype; TABLE 1; FIG. 1, in bold). According to the NCBI Blastn identity, the ITS sequence data indicate that all of our specimens of *Usnea* belong to the subgenus *Eumitria*, and are close to reference sequences of *Eumitria firmula* (MW267133, 92.59%), *Usnea baileyi* (MN080242, 92.78%), and *Eumitria* cf. *pectinata* (MW267149, 91.98%).
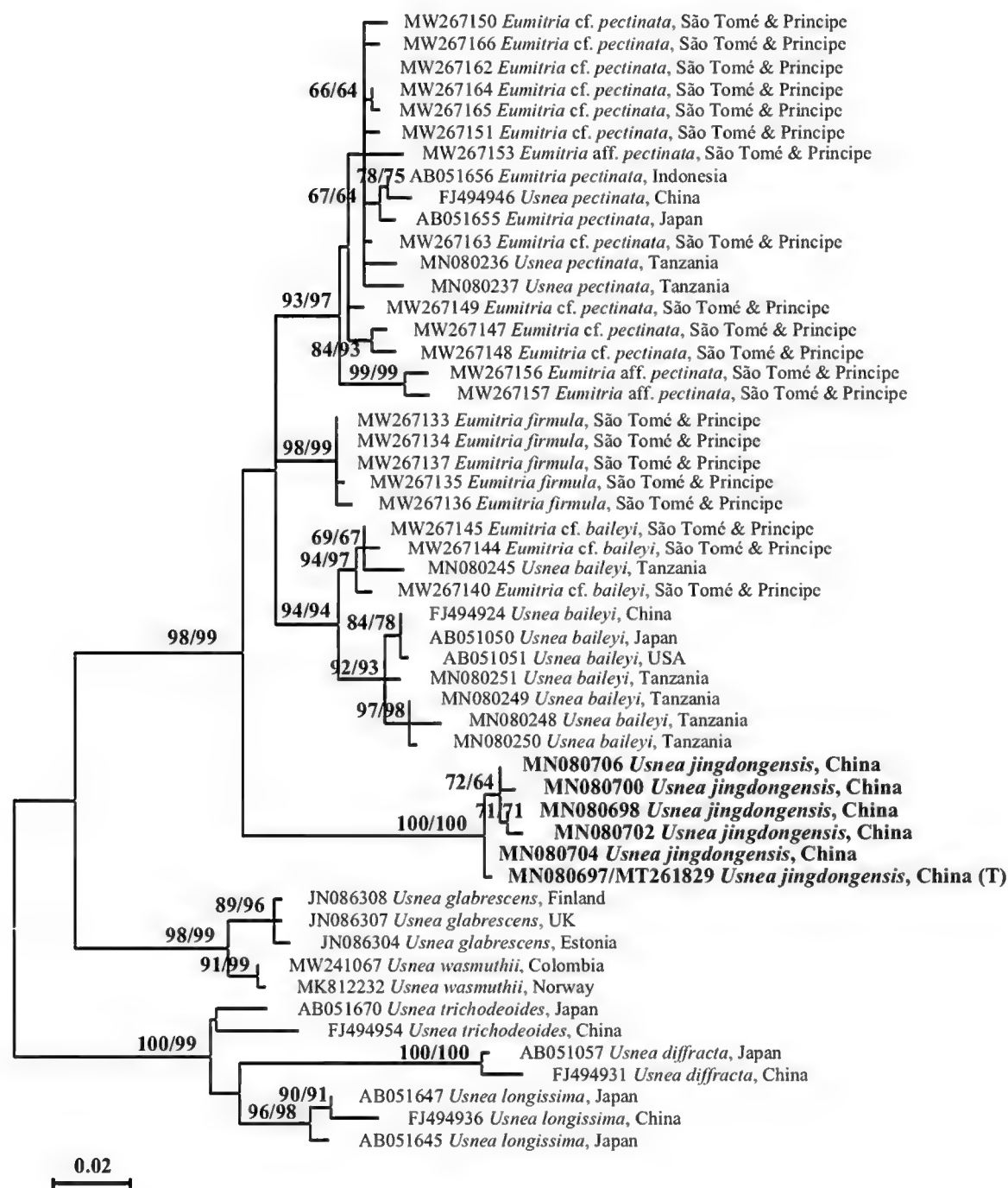

FIG. 1. Phylogenetic relationships of *Usnea jingdongensis* and similar species in *Usnea* subg. *Eumitria*, inferred from nrDNA ITS (ITS1+5.8S+ITS2) sequence data. Species in *U.* subg. *Dolichousnea* were selected as outgroup. Support is indicated for branches characterized by bootstrap frequencies ≥50% with K2+G / GTR+G+I models. Our new sequences are in bold.

The Maximum Likelihood (ML) tree inferred from the nrDNA ITS with the highest log likelihood (−2351.33) is presented in Fig. 1. The sequence data matrix comprised 52 samples (from nine taxa) and 508 characters, of which 355 (69.88%) were constant and 116 (22.83%) were parsimony-informative. All samples of *Usnea jingdongensis* were well defined as a distinct monophyletic lineage with 100% bootstrap support frequencies (BS) by both the Kimura 2+G and GTR+G+I models (BS: 100%/100%). The ML tree of our aligned matrix also recovered our new samples in a strongly supported sister relationship (BS: 98%/99%) with the samples of related species including *U. baileyi* (BS: 94%/94%), *U. firmula* (BS: 98%/99%), and *U. pectinata* (BS: 93%/97%), which all belong to subgenus *Eumitria*.

The results indicated that sequences of nrDNA ITS were sufficient to distinguish all species in the current data set of *Usnea*.

Our proposal of *Usnea jingdongensis* as a new species is justified by its demonstration as a distinct monophyletic clade.

Taxonomy

Usnea jingdongensis S.Y. Guo & L.F. Han, **sp. nov.**   Fig. 2
FN570779

> Differs from *Usnea flaveola* by its thallus trunk jet black at the base, its white or pale brown to brown medulla, its white to pale brown fistulose axis, its tubular axis with loose, pale yellow or pale brown to brown hyphae inside, and its production of protocetraric acid.
>
> Type: China. Yunnan Province, Jingdong county, Ailaoshan Mountain, 24.53°N, 101.02°E, alt. 2450 m, on bark, 5 December 2018, L.F Han & H.D. Wen 2018120543 (**Holotype**, HMAS-L; GenBank MN080697, MT261829).
>
> Etymology. The epithet refers to Jingdong County, where the species was collected.

Thallus corticolous, fruticose, erect or subpendent, up to 11 cm long, greyish-green to yellowish-green when fresh, greyish-green when dry, with anisotomic-dichotomous, sparse to dense ramification (Fig. 2A). Trunk always thinner at the contact point and thickening upwards, with jet black pigmentation extending to the base of the branches, not annulated, up to 1.0 cm long. Main branches 1.0–1.8 mm in diameter, slowly tapering in longitudinal section, terete in cross-section, uninflated. Lateral branches not constricted at the ramification point; foveolae and maculae absent; fibrils slender (up to 5 mm long), abundantly distributed on the branches (Fig. 2B-D); fibercles rare; papillae present on main and lateral branches, many to numerous, short verrucose to cylindrical (Fig. 2B–D); tubercles (containing medulla), numerous, cylindrical (Fig. 2B–D); soralia and isidiomorphs absent.
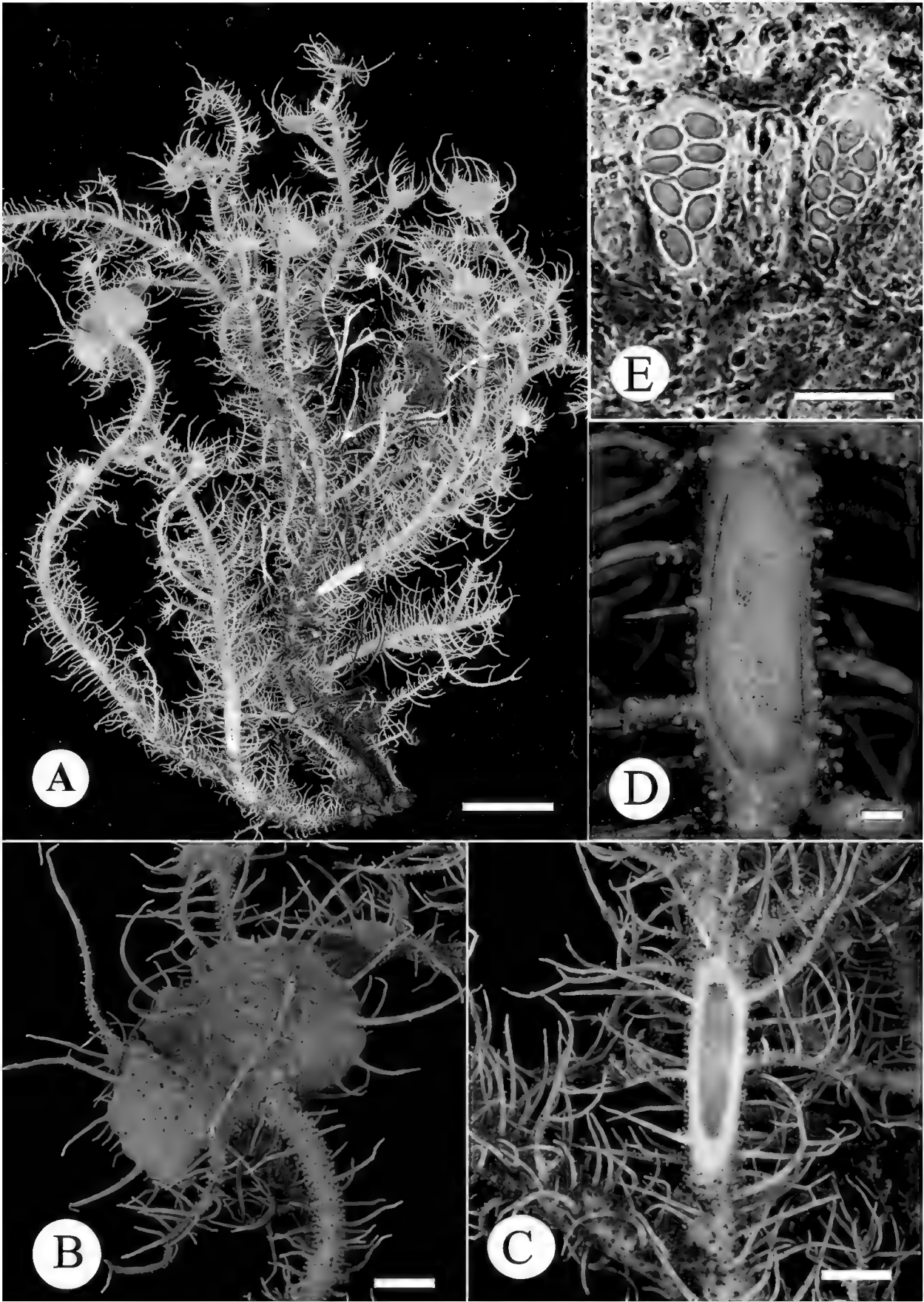
Fig. 2. *Usnea jingdongensis* (A–C, E = holotype, Han & Wen 2018120543; D = Han & Wen 2018120566). A. thallus; B. the reverse side of apothecia; C. fistulose axis with brown loose hyphae inside; D. medulla with brown pigment; E. asci and ascospores. Scale bars: A = 10 mm; B, C = 2 mm; D = 500 μm; E = 20 μm.

CORTEX shiny to vitreous in section, very rigid, lacking pigment, thin to moderately thick, 9–16% of the radius. MEDULLA compact in density, thin, 3–5% of the radius, white or with pale brown to brown pigmentation. FISTULOSE (TUBULAR) AXIS extremely large, axis pale white to pale brown, 79–87% of the diameter; fistulose 36–58%, with loose hyphae inside, pale yellow, pale brown to brown (FIG. 2C, D).

PHOTOBIONT *Trebouxia.*

APOTHECIA common, lateral, up to 10 mm in diameter, disc-shaped, usually flat, with many long cilia and short branches. DISC pale white to pale yellow or brown, not pruinose. PARAPHYSES simple, septate, ± swollen at the apices; EPIHYMENIUM 12.0–18.0 μm thick; HYMENIUM 48.0–70.0 μm thick; HYPOTHECIUM 45.0–75.0 μm thick; ASCI clavate; ASCOSPORES, broad ellipsoid, 8.5–12.5 × 4.5–8.5 μm (FIG. 2E).

PYCNIDIA not seen.

CHEMISTRY—K+ pale yellow, C–, KC+ yellow, Pd+ brick-red. Usnic acid, protocetraric acid, zeorin and three unidentified substances (TLC).

ECOLOGY & DISTRIBUTION—*Usnea jingdongensis* is known only from the type locality where it is abundant with epiphytic lichens in the tropical and subtropical montane forest ecosystems. More than 60 epiphytic lichen species belonging to 26 genera and 17 families were recorded in the primary and secondary forests including six species in *Usnea* (Li & al. 2007; Han & al. 2020).

VARIATION—The abundance of fibrils and papillae may vary among individuals, but both are conspicuously present, at least on the main branches. The colours of medulla, axis, and tubular axis also vary among individuals or in response to environmental changes (FIG. 3).

SELECTED ADDITIONAL SPECIMENS EXAMINED: (All in HMAS-L and HBNU). **CHINA, YUNNAN PROVINCE, Jingdong County**, Mt. Ailaoshan, 24.53°N 101.02°E, alt. 2450 m, on bark, 23 August 2018, L.F. Han 2018082322a (GenBank MN080706, medulla brown, axis white, tubular axis pale brown, FIG. 3D); 5 December 2018, L.F. Han & H.D. Wen 2018120532 (GenBank MN080702); 5 December 2018, L.F. Han & H.D. Wen 2018120562b (GenBank MN080698, medulla grayish white, axis pale brown, tubular axis grayish brown, FIG. 3C); 5 December 2018, L.F. Han & H.D. Wen 2018120566 (GenBank MN080704; medulla with obviously brown pigment, FIG. 2D); 5 December 2018, L.F. Han & H.D. Wen 2018120582 (GenBank MN080700, medulla and axis white, tubular axis brown, FIG. 3B); 23 September 2020, L.F. Han & H.D. Wen 2020092310 (medulla and axis dark brown, tubular axis brown, FIG. 3F); 23 September 2020, L.F. Han & H.D. Wen 2020092316 (medulla, axis, and tubular axis all white to pale white, FIG. 3A); 18 December 2020, L.F. Han & H.D. Wen 2020121815 (medulla dark brown, axis brown, tubular axis faint yellow, FIG. 3E).
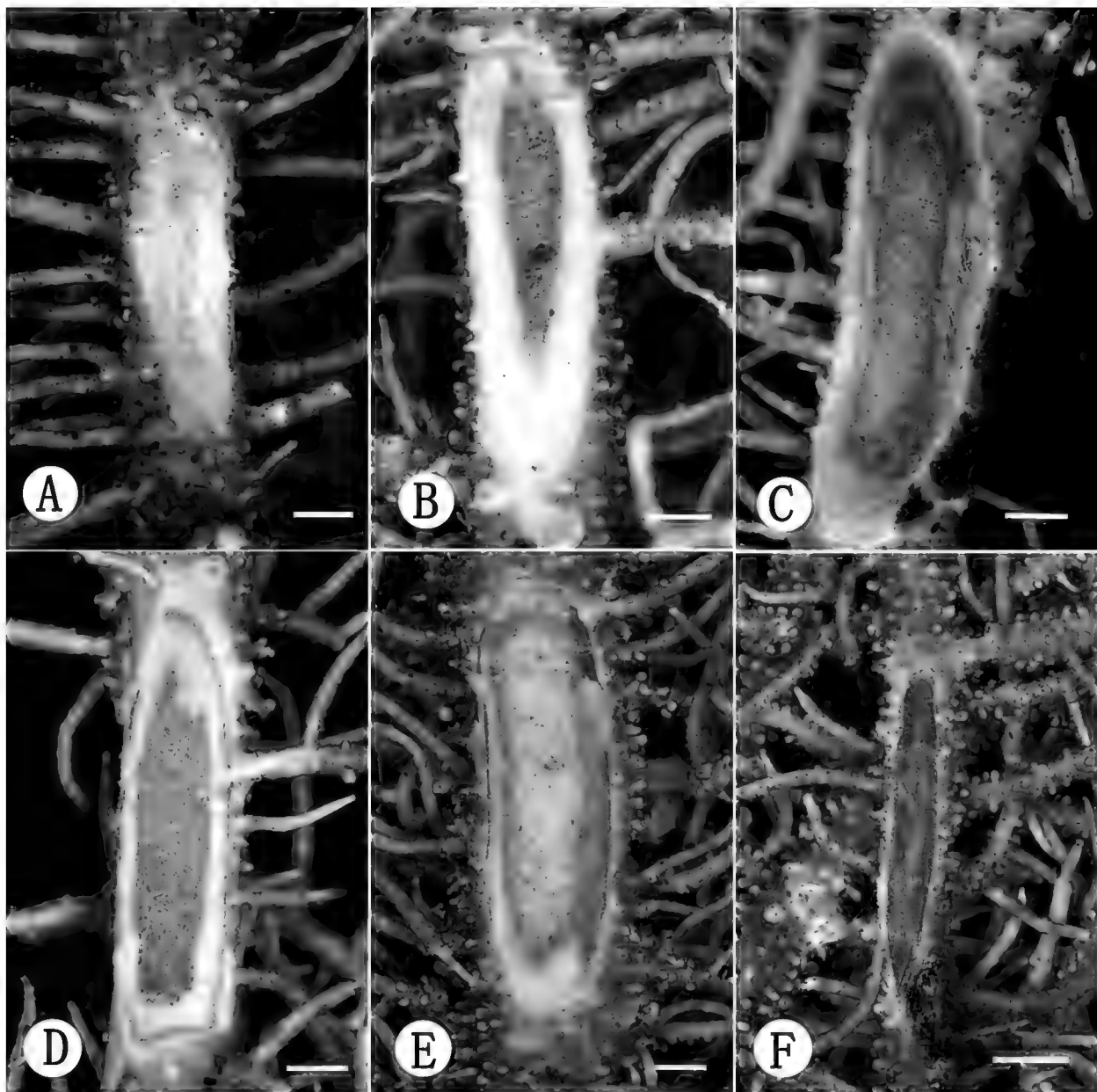
Fig. 3. *Usnea jingdongensis*: colour of medulla, axis, and tubular axis in longitudinal section of branch at ITS widest diameter above first ramification. A. Han & Wen 2020092316, medulla, axis, and tubular axis all white to pale white; B. Han & Wen 2018120582, medulla and axis white, tubular axis brown; C. Han & Wen 2018120562b, medulla grayish white, axis pale brown, tubular axis grayish brown; D. Han 2018082322a, medulla brown, axis white, tubular axis pale brown; E. Han & Wen 2020121815, medulla dark brown, axis brown, tubular axis faint yellow; F. Han & Wen 2020092310, medulla and axis dark brown, tubular axis brown. Scale bars: A, B, D, E = 500 μm; C, F = 750 μm.

Comments—*Usnea jingdongensis* is an eumitrioid *Usnea* species characterized by the fruticose, erect or subpendent thallus with anisotomic-dichotomous branching; jet black trunk base; uninflated branches with numerous fibrils; loose hyphae in fistulose axis with pale yellow to brown pigmentation; and the absence of soralia.

Usnea flaveola Motyka is similar to *U. jingdongensis* in having fistulose axis

and many fibrils, lacking soralia, but differs by the trunk concolorous with the branches, colorless medulla, and the tubular axis with colorless loose hyphae inside (Truong & Clerc 2013).

Usnea pectinata Taylor resembles *U. jingdongensis* in morphology, but is discriminated by a pendulous thallus, sometimes with soralia, pale punctiform maculae on lateral branches, and dark brown pigmented solid axis with some fistulose areas in the central part of the axis in main branch (Ohmura 2001, 2012; Temu & al. 2019). *Usnea jingdongensis* is somewhat related to *U. baileyi* (Stirt.) Zahlbr., which is characterized by the punctiform soralia on the branches, the red pigment in the medulla and the presence of zeorin (Ohmura 2001, 2012; Truong & Clerc 2013; Temu & al. 2019).

Usnea firmula (Stirt.) Motyka is also similar to *U. jingdongensis*, but can be distinguished by being annularly cracked at the base showing the rose medulla, branches slightly inflated, and sparse cilia on the margin of apothecia (Dodge 1956, 1957), and with fibrils numerous along all branches, 1–2 cm long (Swinscow & Krog 1974).

Usnea jingdongensis perhaps resembles *U. brunnescens* C.W. Dodge, which can be distinguished by the thallus being faint brown to russet in the herbarium and producing single isidia on the verrucae (Dodge 1956, 1957).

Morphologically, the new species is closely similar to *Usnea pulvinulata* C.W. Dodge and *U. trullifera* Nyl. in having esorediate and uninflated branches, but *U. pulvinulata* can distinguished by thallus smaller, up to 5 cm, annularly cracked and subareolate in the lower blackened portion; apothecia larger, up to 15 mm in diameter, disc with white-pruinose when young; containing salazinic acid as constant substance (Dodge 1956, 1957; Swinscow & Krog 1974); and *U. trullifera* can be distinguished by the thallus sparsely branched, branches with strongly tuberculate, medulla pale rose next to the axis, and apothecia with a few fibrils along the margin, disc cinnamon with slightly white-pruinose (Motyka 1936; Dodge 1957; Swinscow & Krog 1986), as well as containing salazinic and norstictic acids (Truong & Clerc 2013).

Our sequences for *Usnea jingdongensis* were mentioned in the study of Lücking & al. (2020) as "yet unnamed species composed of unpublished sequences from China (deposited by S. Guo & al. in 2019 and L. Han & al. in 2020)". They demonstrated that the strongly supported clade *Eumitria* formed four supported subclades based on the ITS data, corresponding to our new species *U. jingdongensis*, as well as *U. firmula*, *U. pectinata* s.lat., and *U. baileyi* s.lat. (Ohmura 2002; Nadel 2016; Jaouen & al. 2019; Temu & al. 2019). Our result is consistent with theirs.

Due to the lack of sequence data, the relationship of *Usnea jingdongensis* with other eumitrioid taxa needs to be further investigated. Here, a key to eumitrioid *Usnea* species from China is provided, for better understanding the new species.

Key to eumitrioid Usnea species from China

1. Soralia absent; apothecia common .... *U. jingdongensis*
1. Soralia present; apothecia rare or absent 2
2. Medulla with red, pink or orange pigment 3
2. Medulla without pigment 7
3. Soralia without isidiomorphs .... *U. vainioi*
3. Soralia usually with isidiomorphs, abundant, especially on thinner branches 4
4. Medulla extremely thin (<5%); the entire medulla with pink-red pigmentation .... *U. baileyi*
4. Medulla thicker (>5%); medulla without pink-red pigmentation 5
5. Medulla pigmentation pink to orange; branch segments terete to ridged .... *U. perplectata*
5. Medulla pigmentation pale red; branch segments terete 6
6. Bases of branches almost at right angles to the main branches; the tops of some branches curved in all directions .... *U. subrectangulata*
6. Bases of branches at acute angles to the main branch; the tops of branches distinctly curved downward, especially upper branches .... *U. recurvata*
7. Branches distinctly ridged; axis fistulose .... *U. himantodes*
7. Branches distinctly terete; axis solid or partially fistulose .... *U. pectinata*

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First reports of *Milesina* and *Triphragmium* from Turkey

ŞANLI KABAKTEPE[*1] & ILGAZ AKATA[2]

[1] *Department of Plant and Animal Production, Battalgazi Vocational School, Malatya Turgut Ozal University, Battalgazi, Malatya, Turkey*

[2]*Ankara University, Faculty of Science, Department of Biology, Tandoğan, Ankara, Turkey.*

* CORRESPONDENCE TO: sanli.kabaktepe@ozal.edu.tr

ABSTRACT—Rust species *Milesina scolopendrii* on *Asplenium scolopendrium* and *Triphragmium filipendulae* on *Filipendula vulgaris* are reported from Melsin, Turkey. These are new generic records from Turkey. The morphological characteristics are described and illustrated based on the collected materials.

KEY WORDS—*Pucciniales*, taxonomy, *Milesinaceae*, *Raveneliaceae*

Introduction

Milesina (*Milesinaceae*) is characterised by white sori and no pigment in all spore stages. Its species are often heteroecious; teliospores are not resting spores, but form during the winter and germinate in the spring; both amphispores and urediniospores are produced by some species (Cummins & Hiratsuka 2003). The genus has 36 species, most of are found in northern temperate regions (Bubner & al. 2019), with eleven species found in Europe, including aecia on fir leaves (Bubner & al. 2019; Kirk & al. 2008).

Triphragmium (*Raveneliaceae*) is distinguished by its three-celled teliospores, with a single pore in each cell and with the pedicel attached to the single basal cell (Cummins & Hiratsuka 2003). *Triphragmium* contains seven species. In the northern hemisphere, three *Triphragmium* species parasitize *Filipendula* hosts (Farr & Rossman 2022).

According to the literature on Turkish rust fungi (Akdeniz & Sert 2021; Bahçecioğlu 2014; Bahçecioğlu & Kabaktepe 2012; Kabaktepe 2015; Kabaktepe

& Bahçecioğlu 2012; Kabaktepe & al. 2015a,b,c, 2016, 2017a,b; Kirbag & al. 2011, Özaslan & al. 2015), 375 species belonging to 28 genera have thus far been recorded from Turkey.

Here we report the first records of *Milesina* and *Triphragmidium* from Turkey.

Materials & methods

Specimens of host species were collected from Bolkar mountains (Mersin) in Turkey during 2013–15. The Flora of Turkey (Peşmen & Chamberlain, 1972) was the main literature used for the identification of the host species. Names of host plants and families are given according to WFO: The World Flora Online (http://www.worldfloraonline.org/). Rust fungi were identified morphologically by using methods of Gäumann (1959), Wilson & Henderson (1966), and Ellis & Ellis (1987). Names of fungi were checked from Index Fungorum (http://www. indexfungorum.org). All studied specimens were deposited in the Herbarium of İnönü University, Malatya, Turkey (INU).

For light microscopy, the fungal specimens were isolated from the plant materials either by scraping, or thin sections with a razor blade and spores were scraped from dried host specimens, mounted in lactophenol, and examined with a light microscope. Digital images were made using a Leica light microscope and an Olympus SZ65 stereomicroscope camera. Image Focus v3 analyzing software was used to measure spores. For scanning electron microscopy (SEM), spores were fixed on stubs by using double-sided adhesive tape, coated with gold, and examined using an EVO 40XVP (LEO Ltd., Cambridge, UK) scanning electron microscope at an accelerating voltage of 20 kV.

Taxonomy

Milesina scolopendrii (Fuckel) Jaap, Fung. Sel. Exs.: no. 571 (1912)   Fig. 1

Spermogonia and aecia not seen. Uredinia hypophyllous, scattered or in groups, between veins, greenish-brownish, covered by the epidermis. Urediniospores 24–55 × 14–27 µm, obovoid, clavate or ellipsoid, wall colorless, 0.5–1.5 µm thick, remotely spinulose, spines up to 3 µm, pores scattered, up to 9, mostly 5–6. Telia and basidia not seen.

SPECIMENS EXAMINED—On *Asplenium scolopendrium* L. (*Aspleniaceae*): **TURKEY: MERSIN: Toroslar**, 3 km from Aslanköy to Fındıkpınarı, 20.Sept.2014, Ş. Kabaktepe & I. Akata 7877 (INU 1284); **Çamlıyayla**, Kadıncık valley, Papaz garden located, 820–850 m a.s.l., 26.Jun.2015, Ş. Kabaktepe & I. Akata 8154 (INU 1296).
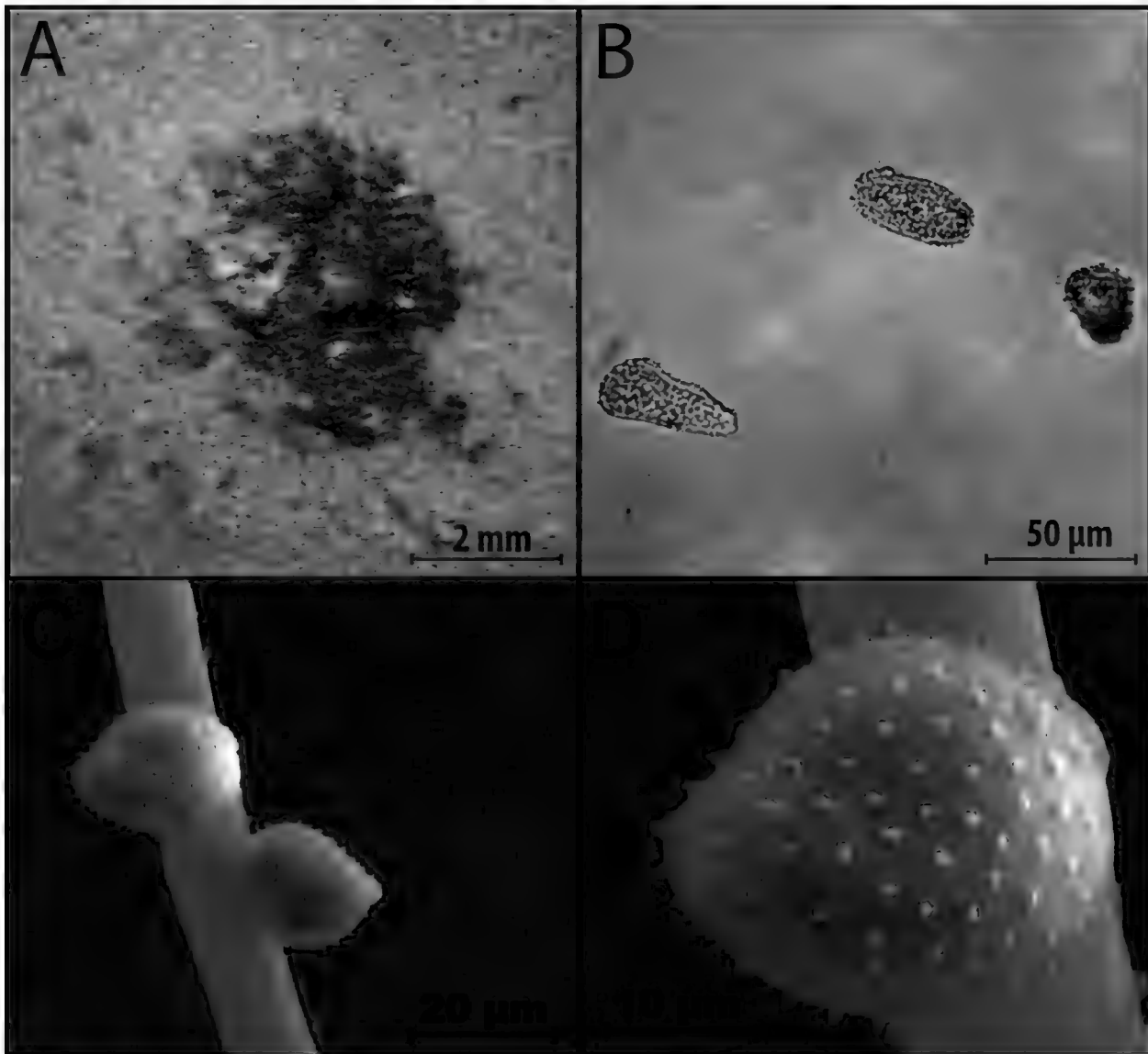
Fig. 1. *Milesina scolopendrii* (INU 1296) on *Asplenium scolopendrium*. A: infected area on leaf tissue; B: urediniospores; C: urediniospores (SEM); D: urediniospore surface (SEM).

Remarks: Although five rust fungi species have been reported on *Asplenium* in Turkey, this is the first Turkish report of a *Milesina* species on that host genus (Bahçecioğlu & Kabaktepe, 2012; Sesli & al, 2020).

Milesina species growing on *Asplenium* are morphologically similar, but can be distinguished from each other by the size, shape, and ornamentation of their urediniospores and by their host species.

Five *Milesina* species have *Asplenium* hosts: *M. asplenii-incisi* (Faull) Hirats. on *A. incisum* Thunb.; *M. feurichii* (Magnus) Grove on *A. septentrionale* (L.) Hoffm.; *M. magnusiana* Jaap on *A. adiantum-nigrum* L.; *M. murariae* (Magnus) Grove on *A. ruta-muraria* L.; and *M. scolopendrii* on *A. scolopendrium*.

M. scolopendrii is characterized by remotely spinulose and large urediniospores (up to 55 µm). It is separated from *M.* asplenii-incisi (up to 26

µm), *M. magnusiana* (up to 35 µm), and *M. murariae* (up to 35 µm) by its larger urediniospores size; and from *M. feurichii* (densely echinulate) by the sparse ornamentation of its urediniospores.

Triphragmium filipendulae (Lasch) Pass., Nuovo Giorn. Bot. Ital.7(3): 255 (1875). FIG. 2
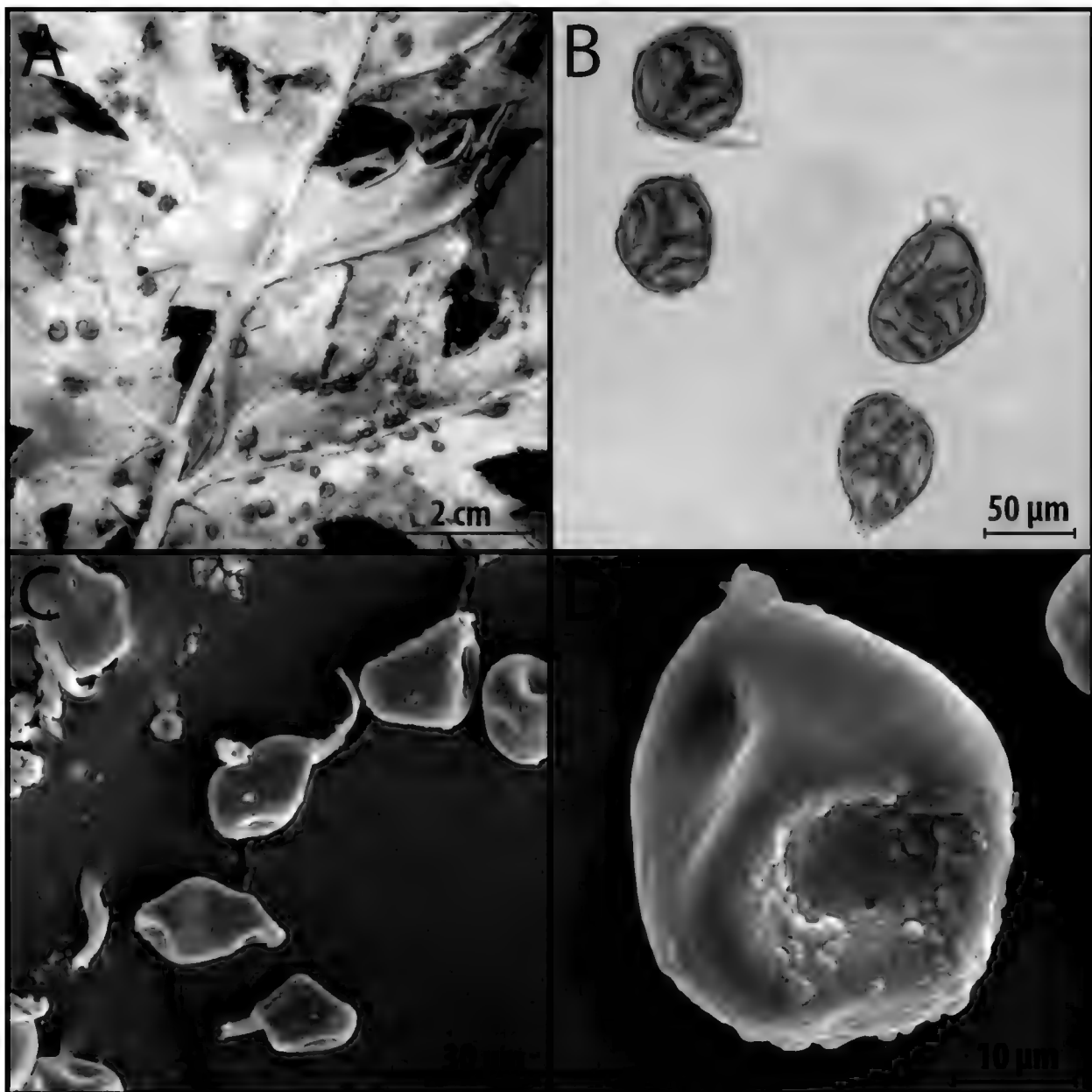
FIG. 2. *Triphragmium filipendulae* (INU 1272) on *Filipendula vulgaris*. A: infected area on leaf tissue; B: teliospores; C, D: teliospores (SEM).

Spermogonia and aecia not seen. Uredinia hypophyllous, scattered, pale orange. Urediniospores 19–30 × 15–21 µm, subglobose, ellipsoid, ovoid, or pyriform, wall pale yellow, up to 2 µm thick, echinulate, pores indistinct. Telia

hypophyllous, scattered, dark brown. Teliospores 28–60 × 18–44 µm, elliptic, scarcely or not constricted at septa, 3-celled (1 basal, 2 apical), each cell with a single pore, brown, smooth or with few warts around the pores, in SEM views small warts especially on pores, pedicel hyaline, equal the spore in length, deciduous. Basidia not seen.

SPECIMENS EXAMINED—On *Filipendula vulgaris* Moench (*Rosaceae*). **TURKEY: MERSIN: Çamlıyayla**, Saydibi plateau, 1800–1880 m a.s.l., 09.July.2013, Ş. Kabaktepe & I. Akata 7345 (INU 1272) ; **Tarsus**, Gülek, Karboğazı, 1700–1800 m a.s.l., 17.Jul.2014, Ş. Kabaktepe & I. Akata 7657 (INU 1285); **Mezitli**, 3 km from Tepeköy to Kocayer, 1140 m a.s.l., 20.Sept.2014, Ş. Kabaktepe & I. Akata 7881 (INU 1286).

REMARKS: Even though three species of *Triphragmium* (*T. filipendulae*, *T. ulmariae* (DC.) Link, and *T. vassilievae* Azbukina) have been reported on *Filipendula* hosts, the genus has not previously been recorded on *Filipendula* in Turkey (Bahçecioğlu & Kabaktepe 2012; Sesli & all., 2020).

Triphragmium ulmariae and *T. vassilievae* are very close to *T. filipendulae* in terms of their similar host and morphology. The smooth cell walls of the teliospores are the characteristic feature of *T. Filipendulae*, while the others have some development of warts on their teliospores.

Acknowledgments

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Laternea triscapa, an enigmatic stinkhorn from the Atlantic Forest of Northeast Brazil

Alisson Plácido-Silva[1], Gislaine C. S. Melanda[2], Iuri G. Baseia[3], Alessandre Pereira-Colavite[4], Felipe Wartchow[4*]

[1] *Universidade Federal da Paraíba, Campus IV, Programa de Pós-Graduação em Ecologia e Monitoramento Ambiental; Rio Tinto, PB, Brazil*

[2] *Universidade Federal de Pernambuco, Departamento de Micologia, Programa de Pós-Graduação em Biologia de Fungos; Recife, PE, Brazil*

[3] *Universidade Federal do Rio Grande do Norte, Departamento de Botânica e Zoologia; Natal, RN, Brazil*

[4] *Universidade Federal da Paraíba, Campus I, Departamento de Sistemática e Ecologia; João Pessoa, PB, Brazil*

[*] Correspondence to: fwartchow@yahoo.com.br

Abstract — A specimen of *Laternea* was found in the Barra do Rio Mamanguape Environmental Protected Area, Paraíba, Brazil. The basidioma is composed of four vertical arms joined at the apex, and it presents a pendant glebifer below the junction. It is described here as an authentic specimen of *L. triscapa* from Northeast Brazil, with photographs and drawings of the basidioma and microstructures, and discussion of the names attributed to the genus.

Key words — *Clathraceae*, neotropics, *Phallales*, *Phallomycetidae*, taxonomy

Introduction

Clathraceae Chevall. is a recently rearranged family that groups the genera *Abrachium* Baseia & T.S. Cabral, *Aseroe* Labill., *Blumenavia* Möller, *Clathrus* P. Micheli ex L., *Ileodictyon* Tul. & C. Tul., *Laternea* Turpin, and *Pseudocolus* Lloyd (Melanda & al. 2021). *Laternea* was originally described to accommodate *L. triscapa,* a species with basidiomata usually characterized by three vertical

arms, joined at the apex with the gleba present only below this junction, differing from *Clathrus* in having a pendant glebifer (Levrault 1822, Linder 1928). Later, Cunningham (1931a, 1931b, 1944) proposed that the genera *Laternea*, *Linderiella* G. Cunn., and *Blumenavia* constituted a validy named tribe *Columnateae* G. Cunn. (see Turland & al. 2018: Art. 39.1) within *Clathraceae* characterized by the receptacle composed of vertical arms joined at the apex. The other two tribes proposed by Cunningham were *Clathrateae* G. Cunn., characterized by the receptacle composed of interconnected arms forming a cage, and *Stellateae* G. Cunn., characterized by pseudostipitate basidiomata with a branched part at the top. Dring (1980) concurred in including *Laternea* as a separate genus from *Clathrus*, differentiated mostly by the form of receptacle and the position of the glebifer. Later, Pegler & Gómez (1994) considered three types of basidiomata in *Clathraceae*, namely the clathroid, lysurioid and laternoid series, with the last of these including the genera *Laternea* and *Linderiella*, differing from the other series by the gleba being confined to the inner part of the joined arms at the top of the basidioma.

The *Laternea* type species, *L. triscapa*, was described from Île de la Tortue, Haiti (Levrault 1822). In Brazil, *L. triscapa*, *L. pusilla* Berk. & M.A. Curtis, and *L. dringii* A. López & al. have been recorded (Rick 1961, Bononi & al. 1984, Meijer 2006, Magnago & al. 2013, Lima & al. 2019, Trierveiler-Pereira & al. 2019). Entities identified as *Laternea triscapa* were cited from Rio Grande do Sul (Rick 1961), and São Paulo (Bononi & al. 1984); however, specimens from Northeast Brazil (Baseia & al. 2006, Leite & al. 2007) were equivocally cited, and actually belong to *Clathrus* sensu Dring (1980) due to the gleba dispersed in the inner surface of the arms, without a pendant glebifer.

Our work aims to report authentic specimen of *Laternea triscapa* from Northeast Brazil, as well as discussing other *Laternea* names reported for this region.

Materials & methods

The specimen was collected in the Área de Proteção Ambiental (APA) Barra do Rio Mamanguape, an area of 14,460 ha located among the municipalities of Rio Tinto, Marcação, and Lucena (6.83°S 34.92°W). The vegetation comprises Atlantic Forest fragments with characteristic flora, including members of *Rubiaceae*, *Fabaceae*, *Anacardiaceae*, *Boraginaceae*, *Euphorbiaceae*, and *Sapindaceae*, among others (Pereira & Alves 2007). Color codes follow Online Auction Color (2004); and morphological studies follow methodologies of Baseia & al. (2014) and Melanda & al. (2020). The material is deposited

in Herbário Lauro Pires Xavier, Universidade Federal da Paraíba, Cidade Universitária, João Pessoa, Paraíba, Brazil (JPB).

Taxonomy

Laternea triscapa Turpin, in Levrault, Dict. Sci. Nat. 25: 248. 1822.   Fig. 1
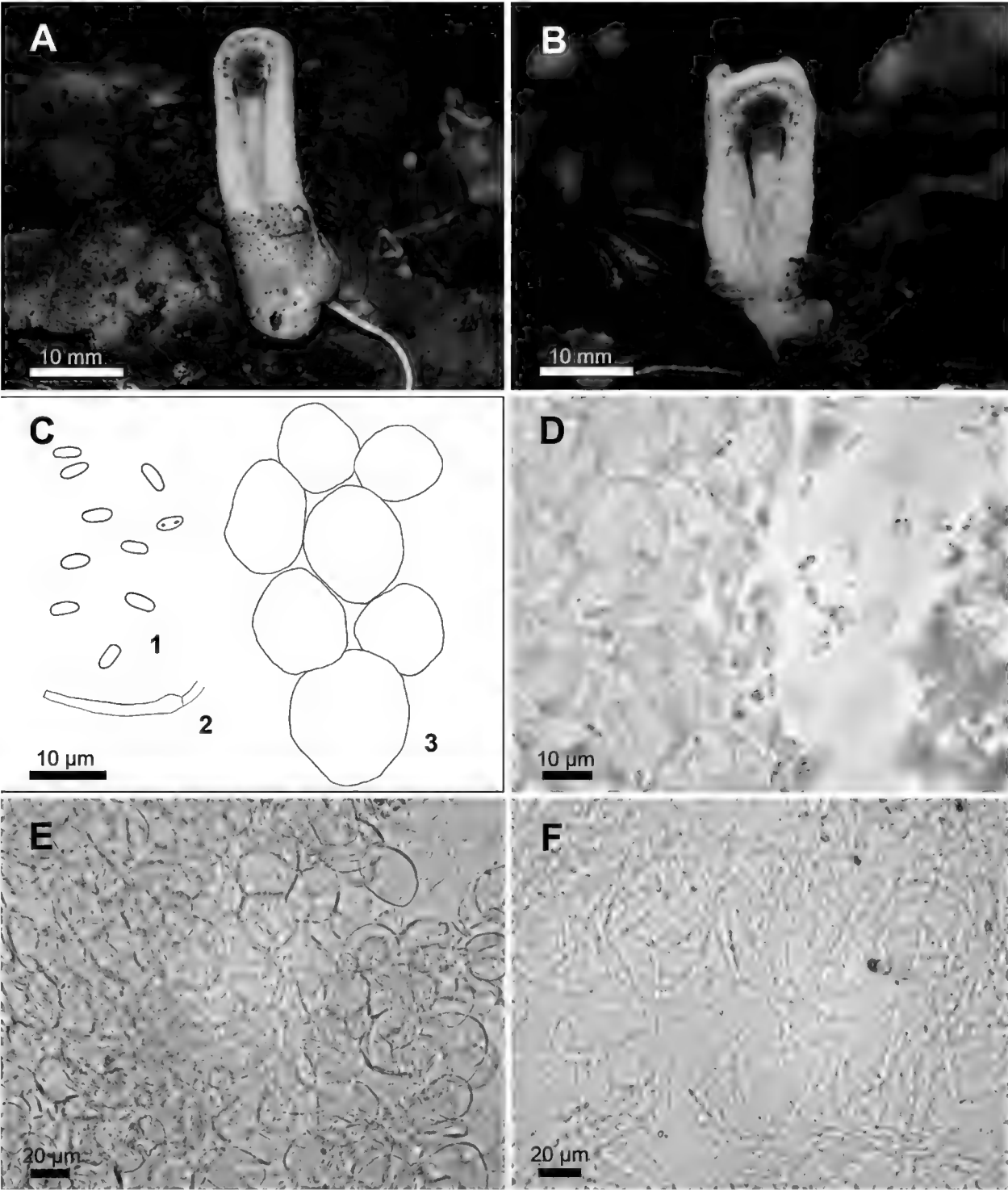

Fig. 1. *Laternea triscapa* (JPB 65670). A, B. Basidioma in situ; C1. Basidiospores; C2. Single volval hypha from the outer grey layer; C3. Elements of the receptacle; D. Basidiospores and adjacent elements of the glebifer; E. Elements of the receptacle; F. Hyphae of the outer grey layer of the volva.

Unexpanded basidiomata not observed. Basidioma small in size, 33 mm high, receptacle erect with four arms, united at apex and free below, with pink-orange color (OAC 651, 652) at apex, paler towards base (paler than OAC 655); each arm is 20 mm high, 3 mm in diam. at base, and 2 mm diam. at apex. Gleba concentrated in an apical salmon-orange (OAC 651, 652) pendant glebifer. Volva very thin, 15 mm high, membranous, whitish (near OAC 909), with greyish brown (OAC 641) appressed dotted squamules. Rhizomorphs present, white (OAC 909).

Basidiospores 4.1–4.6 × 1.5–2.0 μm, x = 4.5 ± 0.4 × 1.5 ± 0.2 μm, Q = 2.0–3.3; Qm = 2.8, hyaline, cylindrical, thin-walled. Receptacle composed of pseudoparenchymatous cells, 12.2–31.3 × 11.7–31.3 μm, (x = 19.2 ± 5.0 × 18.0 ± 6.4 μm, d = 0.7–1.5; Qm = 1), hyaline. Glebifer composed of pseudoparenchymatous cells similar to receptacle ones. Gleba has a mass of basidiospores. Volva of outer grey layer comprising septate hyphae 36.2–61.2 × 1.5–3.1 μm (x = 47.3 ± 7.9 × 2.3 ± 0.7 μm), hyaline, sometimes slightly swollen near septa, thin to very occasionally slightly thick-walled ≤0.5 μm. Clamp connections absent from all observed septa.

SPECIMEN EXAMINED—**BRAZIL, PARAÍBA, Rio Tinto,** APA Barra do Rio Mamanguape, Mata do Oiteiro, 7.viii.2019, F. Wartchow FW 53/2019 (JPB 65670).

HABIT AND HABITAT: solitary, on soil among litter, in an Atlantic Forest fragment in Northeast Brazil.

CONSERVATION STATUS—This material of *L. triscapa* was collected in a conservation unit with a sustainable purpose (Cabral & al. 2009). Due to the impossibility of defining the population and consequent lack of clear data on abundance and distribution in the locality, we consider this taxon to belong to the category of 'Data Deficient' (DD), but more information is required to evaluate it according to the IUCN criteria (IUCN 2019).

Discussion

Our material is characterized by the expanding basidioma 33 mm high, pink-orange above, paler below, and receptacle presenting four arms without crests. In the volva a grayish-dotted squamules on a whitish ground was observed in the field. The specimen is somewhat similar to *Laternea dringii* sensu Lima & al. (2019), from Ceará, Brazil, in basidiomata height (26–40 mm) and basidiospores measurement (3.5–4.6 × 1.1–2 μm), but differing only in the orange tonalities of the arms (according to Küppers color atlas used by Lima & al.). The same orange tones occur in specimens identified as *L. dringii* from Paraíba, Brazil (Magnago & al. 2013) and from Veracruz, Mexico (López & al.

1981, López & García 2001), which have smaller basidiomata up to 15 mm high, and (for the Mexican specimens) basidiospores slightly longer and narrower: 4.2–5(–5.6) × 1.4 µm; Magnago & al. (2013: 238) did not measure basidiospores because, according to them, their 'voucher specimen is not preserved'.

The concept for generic placement of *Laternea dringii* by López & al. (1981) into the genus *Laternea* is not supported by the type specimen; the arms were described as red then gradually discoloring to orange then yellow at the base and the gleba is inserted in the lower region of the arms then spreading toward the apex. The protologue (López & al. 1981: 111) and the 'Plate 1' containing 'Figures 1-2' (López & al. 1981: 114) strongly suggest a clathroid fungus (sensu Pegler & Gómez 1994, Ribeiro et al. 2022), probably belonging in *Clathrus* close to *C. columnatus* Bosc, as originally described by Bosc (1811). This was contradicted in the record of the species later published by López & García (2001), who showed a pendant glebifer characteristic of *Laternea*. Unfortunately, there is no available DNA from the type specimen of *L. dringii* to confirm this hypothesis by phylogeny. However, based on its morphological characteristics, we have no doubt that López & al. (1981: 111) described a clathroid fungus. Thus the identity of Brazilian specimens labelled as *Laternea dringii* must be rejected.

Laternea pusilla, originally described from Cuba (Berkeley 1868) has similar basidiomata size to our specimen of *L. triscapa* (20–45 mm tall). However, the Cuban species differs in the reddish arms with crested projections (Sáenz 1976, Sáenz & al. 1983, Calonge & al. 2005, Sandoval-Leiva & al. 2014, Pinzón-Osorio & al. 2017).

Laternea triscapa was defined by Turpin (Levrault 1822: 248–249) as being similar to *Clathrus*, but having a structure described by him as 'conceptacle en forme cul-de-lampe ou de houppe' adhered in a vault produced by the junction of the superior portions of the three arms. In addition, the basidioma was described as measuring two inches (up to 50 mm) high and equally two inches wide in the expanded fungus, and the color was indicated as a 'beautiful vermilion red'.

Since many clathroid species were frequently included in the genus *Laternea*, Linder (1928) and Cunningham (1931, 1944) restored the original concept of Turpin (Levrault 1822). In addition, Linder (1928) collected his material in a sugar cane plantation in Cuba and reported basidioma ranging from 50–62 mm high, 'capucine buff' (= light orange-yellow, moderately orange-yellow, pale orange-yellow, according to Kelly & Judd 1976) at base then 'cadmium orange' (= vivid orange, strong orange, brilliant orange, Kelly & Judd 1976)

toward the apex, bearing three columns, 'nopal red' (= strong red, Kelly & Judd 1976) angled glebifer, and whitish volva. Dennis (1953) also reported the specimens under this name from Trinidad with orange tints.

Later, Dring (1980) performed the most complete revision of *Laternea triscapa*, analyzing materials from Cuba, Haiti, Puerto Rico, Trinidad & Tobago, Belize, and Chile. He found basidiomata up to 70 mm high, vermilion above then pinkish below, three or occasionally four columns, bright reddish orange glebifer, and white egg with sepia stain.

There are other studies citing specimens under this name: Berkeley (1868) reporting a 'pale vermillion' color for Cuban specimens; Rick (1961) reported from South Brazil, with cinnabar receptacle apex and whitish base; Dumont & Umaña (1978) referred to the receptacle as red above and white below in Colombian specimen; and Bononi & al. (1984) reported from Southeast Brazil with receptacle composed of three arms joined at the apex. However, none of them mentioned the presence of a glebifer and the basidiomatal color and shape. Finally, *Laternea triscapa* sensu Baseia & al. (2006) and Leite & al. (2007) clearly corresponds to *Clathrus*, due to the lack of a pendant glebifer.

Thus although difference in the basidioma color, but based in other characteristic mentioned by Turpin (Levrault 1822), Linder (1928), Dennis (1953), and Dring (1980), we consider our specimen as authentic *Laternea triscapa* collected in Brazil, and the most satisfactory defined fungus in its original concept.

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MYCOTAXON

New records of lichenicolous and lichenized fungi from Üzümdere Nature Park, Türkiye

Mustafa Kocakaya[1*], Mehmet Ünsal Barak[1], Zekiye Kocakaya[2]

[1] *Department of Organic Agriculture, Boğazlıyan Vocational School*
    University of Yozgat Bozok, Yozgat, Türkiye
[2] *Department of Crop and Animal Production, Safiye Cikrikcioglu Vocational School*
    Kayseri University, Kayseri, Türkiye
* Correspondence to: mustafa.kocakaya@yobu.edu.tr

Abstract — Four species of lichenicolous fungi (*Clypeococcum epicrassum, Polycoccum cladoniae, Sphaerellothecium arnoldii,* and *Stigmidium* aff. *lecidellae*) and two species o f lichenized fungi (*Gyalolechia klementii* and *Parvoplaca servitiana*) are reported for the first time from Turkey. Comments on their morphological characters, habitats, and substrates are provided, together with macro- and microphotographs.

Key words — biodiversity, lichens, *Mycosphaerellaceae, Polycoccaceae, Teloschistaceae*

Introduction

Lichenized and lichenicolous fungal research in Turkey has increased considerably in the last 30 years. According to published floristic studies for various regions of Turkey, c. 1900 species of lichenized fungi are known from Turkey (John & Türk 2017; John & al. 2020). Numerous studies of lichenicolous fungi have been conducted recently (Hafellner & John 2006; Halıcı 2008; Halıcı & al. 2012; Kocakaya & al. 2015, 2016, 2018, 2020; John & al. 2020; Kocakaya 2021), resulting in about 200 lichenicolous fungi species recorded from Turkey.

In Turkey, Üzümdere Nature Park is located in the Middle Taurus Mountains between Konya and Antalya provinces. The study area has altitudes 247–1877 m a.s.l. (with Ulusivri Hill as the highest elevation). The different geomorphological features of the area and the humid environments created by

the Manavgat Stream create a wide range of different ecosystems and habitats in the study area, including forested ecosystems and rocky vegetation. Üzümdere Nature Park is under the influence of the Mediterranean climate in terms of natural vegetation (Uyar 2018).

Pinus brutia forest communities also include other trees from maquis elements, such as *Pistacia terebinthus* subsp. *palaestina, Olea europaea* var. *europaea, Laurus nobilis,* and *Arbutus andrachne;* and shrubs of *Calicotome villosa, Daphne sericea, Nerium oleander, Quercus coccifera,* and *Styrax officinalis* are found (Demirelma & Ertuğrul 2009). With so many different tree species and habitat diversity in the study area, we believe that there will be many more lichen and lichenicolous fungi to be found in future studies.

The aim of this paper is to contribute to knowledge of the lichen and lichenicolous fungi biodiversity of Turkey.

Material & methods

All species were collected from Üzümdere Nature Park (Antalya/Konya) during 2019–20. The specimens are stored in the Lichen Herbarium of Yozgat Bozok University (Science and Art Faculty), Yozgat, Turkey (UZD). The species were examined in Lugol's solution and KOH (10%) in adition to water, but spore measurements were made in KOH. All specimens were examined with a stereomicroscope (Olympus SZX16) and a light microscope (Olympus BX53). Macro- and microphotographs were taken with a digital microscope camera (Olympus DP72).

Taxonomy

Four species of lichenicolous fungi (*Clypeococcum epicrassum, Sphaerellothecium arnoldii, Polycoccum cladoniae,* and *Stigmidium* aff. *lecidellae*) and two taxa of lichenized fungi (*Gyalolechia klementii* and *Parvoplaca servitiana*) are reported as new records for Turkey. Each species is annotated with ecological data, and comparisons with published descriptions of the same species and with related species.

New records of lichenicolous fungi

Clypeococcum epicrassum (H. Olivier) Hafellner & Nav.-Ros., Bull. Soc. Linn. Provence 45: 423 (1994)   Fig. 1A,B

Ascomata immersed, perithecioid, arising in groups often causing necrotic patches on the host thallus; 90–150 μm; wall dark brown, 10–11 μm. Hymenium colourless, I–. Asci 50–85 × 14–19 μm, (4–)8-spored. Ascospores ellipsoidal, brown, 1-septate, slightly constricted at the septum, 14–22 × 6–10 μm (n = 30).
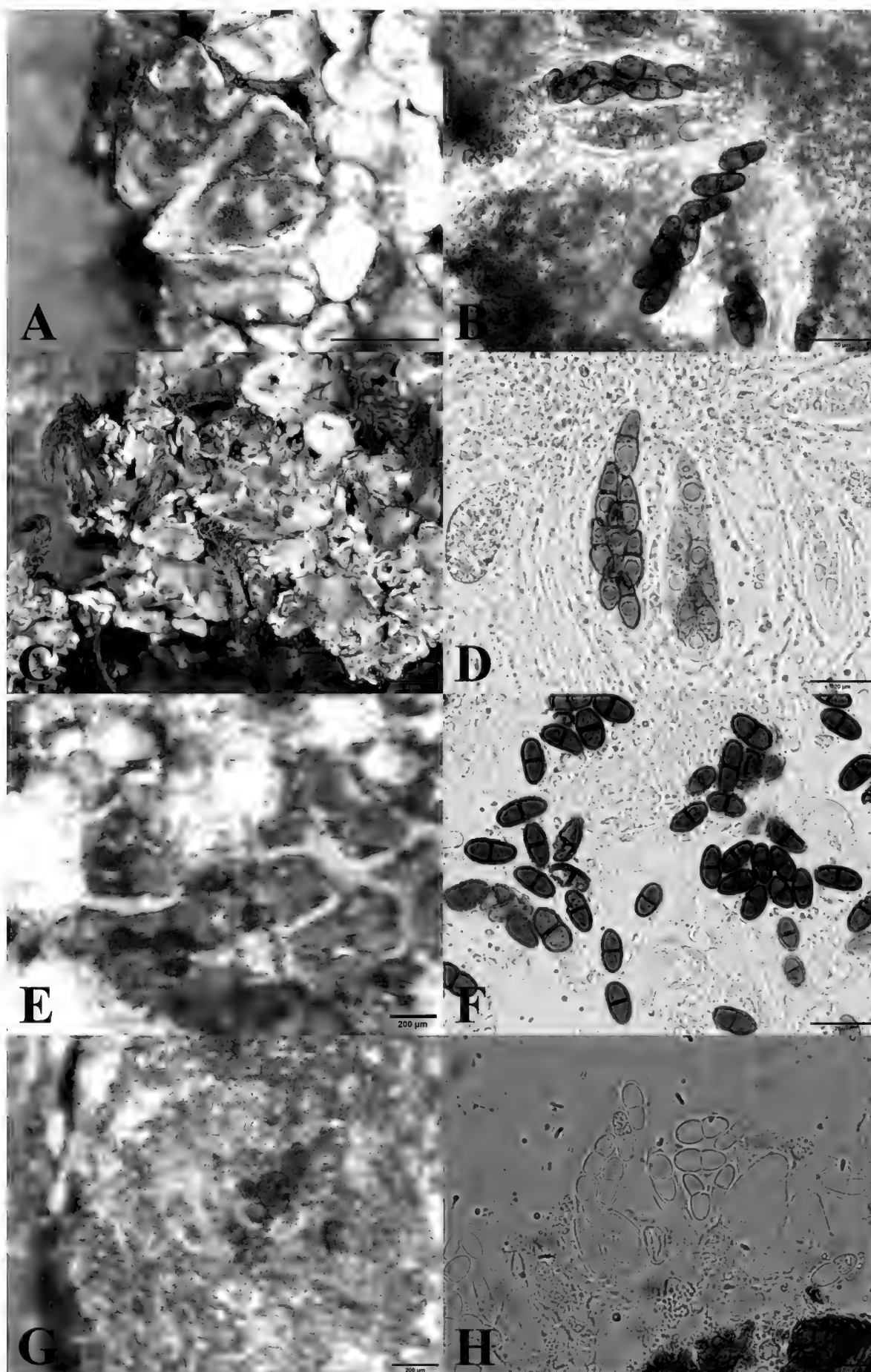
FIG. 1. *Clypeococcum epicrassum* (UZD 0.633) on *Squamarina cartilaginea*: A. Perithecia; B. Ascus and ascospores. *Polycoccum cladoniae* (UZD 0.632) on *Cladonia pyxidata*: C. Perithecia; D. Ascus and ascospores. *Sphaerellothecium arnoldii* (UZD 0.077) on *Lecania rabenhorstii*: E. Perithecia; F. Ascus and ascospores. *Stigmidium* **aff.** *lecidellae* (UZD 0.605) on *Lecidella elaeochroma*: G. Perithecia; H. Ascus and ascopores.

SPECIMEN EXAMINED—**TURKEY. ANTALYA, İbradı**, Akseki–İbradı Road, 37.0459°N 313634°″E, 980 m, on thallus of *Squamarina cartilaginea* (With.) P. James, 14 September 2019, M. Kocakaya & M.U. Barak (UZD 0.633).

COMMENTS— Our Turkish specimen's characters are coherent with the description given by Navarro-Rosines & al. (1994). According to Navarro-Rosines & al. (1994), this lichenicolous fungus has become widespread in Mediterranean and arid regions and it grows in the thallus of several species of *Squamarina*. This taxon causes clear spots in host thallus but apparently does not cause serious damage.

Only one *Clypeococcum* species, *C. hypocenomyces* D. Hawksw., has been previously reported from Turkey (Hafellner & John 2006; Halıcı & Candan 2007), growing on *Hypocenomyce scalaris* (Ach.) M. Choisy.

DISTRIBUTION— *Clypeococcum epicrassum* has been previously reported from France, Greece (Navarro-Rosines & al. 1994), and Spain (Navarro-Rosines & al. 1994; Hafellner & Porcel 2003).

Polycoccum cladoniae Diederich & D. Hawksw., Trans. Brit. Mycol. Soc. 90(2): 297 (1988)   FIG. 1c,d

Ascomata perithecioid, superficial, subglobose, ostiolate, black, 85–130 μm diam., wall dark brown, thickened near the ostiole, cells pseudoparenchymatous, 2–6 μm. hamathecium consisting of branched and anastomosing septate pseudoparaphyses. Asci clavate, bitunicate, thick-walled,containing 8 spores, 45–76 × 15–19 μm. Ascospores 1-septate, brown, verrucose, constricted at the septum, 16.5–21 × 5–8 μm (n = 30)

SPECIMEN EXAMINED—**TURKEY. ANTALYA, İbradı**, Akseki–İbradı Road, 37.0832°N 31.6096°E, 980 m, on squamules of *Cladonia pyxidata* (L.) Hoffm., 14 September 2019, M. Kocakaya & M.U. Barak (UZD 0.632).

COMMENTS— *Polycoccum cladoniae* is separated from other *Polycoccum* species by the size and shape of the ascomata and ascospores: ascomata 100–250 μm diam.; asci elongate-clavate, 40–60(–80) × 9–12(–14) μm; and ascospores ovoid, 1-septate, 13.5–16.5 × 6.5–8 (Hawksworth & Diederich 1988). The ascospores of our Turkish specimen are longer than in the original description (Hawksworth & Diederich 1988).

Polycoccum cladoniae is most similar to *P. microcarpum* Diederich & Etayo, which is also known to occur on *Cladonia* and with ornamented ascospores, but *P. cladoniae* has larger, more immersed ascomata, longer asci, and longer ascospores (Hawksworth & Diederich 1988).

DISTRIBUTION— *P. cladoniae* has been previously reported from Germany and Austria (Hawksworth & Diederich 1988).

Sphaerellothecium arnoldii (A. Massal.) Hafellner, Fritschiana 94: 27 (2019)

FIG. 1E,F

≡ *Tichothecium arnoldii* A. Massal., Misc. Lichenol.: 27 (1856).

= *Phaeospora arnoldii* Hepp, Flechten Eur.: no. 701. (1860).

≡ *Polycoccum arnoldii* (Hepp) D. Hawks., Bot. Not. 132(3): 289. (1979).

Ascomata 80–100 μm., superficial, globose. Hamathecial filaments 1.5–2.5 μm thick. Asci elongate clavate, 8-spored. Ascospores ellipsoid, 1-septate with unequal cells, brown, 12–16 × 5.5–7 μm (n = 25).

SPECIMEN EXAMINED—**TURKEY. ANTALYA, Akseki**, Üzümdere, Üzümdere Village Road, 37.1318°N 31.7002°E, 650 m, on thallus of *Lecania rabenhorstii* (Hepp) Arnold, 13 September 2019, M. Kocakaya & M.U. Barak (UZD 0.077).

COMMENTS— *Sphaerellothecium arnoldii* has been reported mostly from *Diploschistes* and *Rhizocarpon* hosts, but also from *Urceolaria*.

Our specimen growing on *Lecania rabenhorstii* has ascospores longer, measurement 12–16 × 5.5–7 μm, and asci wider, measurement 30–50 × 13–20 μm, than those reported by Hawksworth & Diederich (1988). The Turkish specimen causes bleaching on host thallus.

DISTRIBUTION— *Sphaerellothecium arnoldii* has been previously reported from UK, Germany, Denmark, France, Luxembourg, Austria, Czechoslovakia, Italy, and Venezuela (Hawksworth & Diederich 1988; Santesson 1993; Berger & Priemetzhofer 2000; Atienza & al. 2003).

Stigmidium aff. lecidellae Triebel, Cl. Roux & Le Coeur, Canad. J. Bot. 73(4): 663 (1995)

FIG. 1G,H

Ascomata black, superficial 60–110 μm. The periphyses, simple, 5–12 × 1–2.5 μm., Asci cylindrical, 8-spored, 40–60 × 15–23 μm. Ascospores 1-septate, colourless, oblong, 14–18 × 5–7 μm (n = 24).

SPECIMEN EXAMINED—**TURKEY. ANTALYA, İbradı**, Akseki–İbradı Road, 37.0832°N 31.6096°E, 980 m, on thallus of *Lecidella elaeochroma*. 14 September 2019, M. Kocakaya & M.U. Barak (UZD 0.605).

COMMENTS—The ascopores of our Turkish material are wider than in the original description (14–18 × 5–7 μm; Roux & al. 1995). *Stigmidium* aff. *lecidellae* was described on the apothecia of *Lecidella elaeochroma* (Ach.) M.

Choisy from France (Roux & al. 1995), having asci with 8 spores, broadly cylindrical or claviform, 22–36 × 8–13 μm, sessile or almost, fissitunicate, thick-walled; and ascospores colorless, (11–)12–13.8–15(–16) × 3–3.3–4 μm, oblong or long ellipsoidal.

Distribution— *Stigmidium* aff. *lecidellae* has previously been reported from France (Roux & al. 1995), Italy (Brackel 2008), and Russia (Urbanavichus & Urbanavichene 2014).

New records of lichenized fungi

Gyalolechia klementii (Kalb) Søchting, Frödén & Arup, Nordic J. Bot. 31(1): 71 (2013)   Fig. 2a,b

Thallus pruinose, distinctly marginal lobes, yellow. Apothecia 0.6–1.4 mm diam, reddish brown, lecanorine. Hymenium up to 75 μm, asci 4-spored, 70–80 × 20–28 μm. Ascospores simple and broadly fusiform, with oil drops, 17–20 × 7–9.5 μm. (n = 30). Thallus and apothecia react with KOH (purple). Pycnidia not observed.
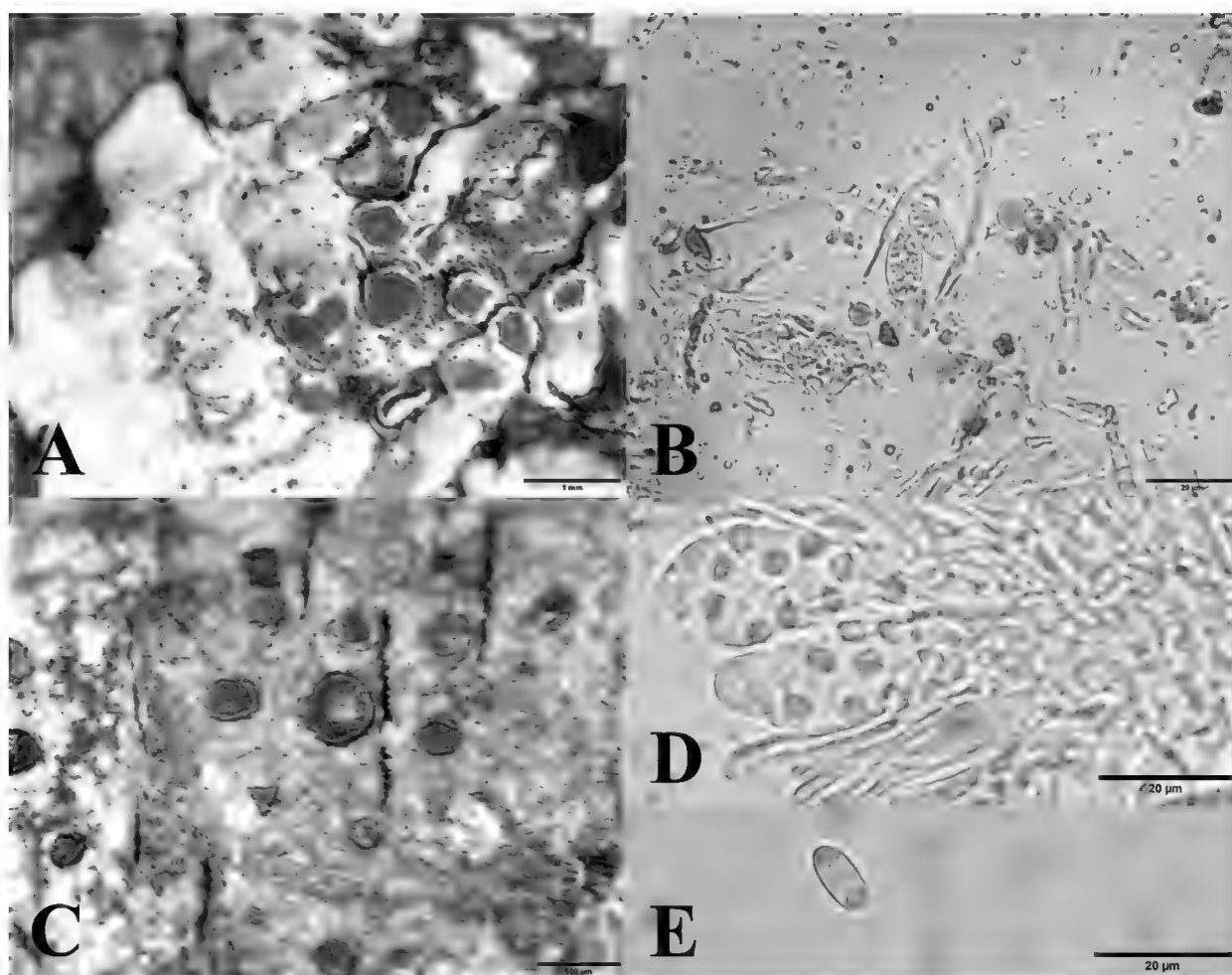

Fig. 2. *Gyalolechia klementii* (UZD 0.356): A. Thallus and apothecia; B. Ascospores. *Parvoplaca servitiana* (UZD 0.444): C. Thallus and apothecia; D. Ascus; E. Ascospore.

Comments — Members of *Gyalolechia* are characterized by chemistry that is dominated by fragilin and commonly a yellow thallus (Arup & al. 2013). Thallus irregular. Apothecia numerous, scattered or contiguous, 0.7–1.3 mm wide. Hymenium 80 μm (Kalb 1970).

Gyalolechia klementii is easily separated from other species in the genus by the pruinose thallus and the ascospore type (Kalb 1970). There are nine species belonging to *Gyololechia* in Turkey (John & Türk 2017).

Distribution— *Gyalolechia klementii* has previously been reported from Spain (Kalb 1970, as *Fulgensia klemetii*) and Greece (Christensen 1995).

Parvoplaca servitiana (Szatala) Arup, Søchting & Frödén, Nordic J. Bot. 31(1): 49 (2013)   Fig. 2c–e

Thallus thin, greyish green, apothecia black, biatorine, 0.2–0.6 mm diam, well-developed true exciple. Hymenium 70–100 μm. Asci broadly cylindrical, 30–40 × 10– 18 μm, 8-spored. Ascospores 13.5–15 × 5–7 μm and septum 5–8 μm (n = 30).

Comments— The species known as *Caloplaca servitiana* was transferred to the genus *Parvoplaca* by Arup & al. (2013). *Parvoplaca servitiana* is similar to *Caloplaca oleicola* (J. Steiner) van den Boom & Breuss but is clearly distinct. *Caloplaca oleicola* has a thin white thallus and biatorine apothecia with prosoplectenchymatous true exciple and without a thalline exciple (Vondrak & al. 2010), while *P. servitiana* has a well-developed true exciple. In the molecular study by Vondrak & al. (2012), *P. servitiana* was not closely related to any known European species with no known closely related lineages.

Distribution—*Parvoplaca servitiana* has previously been reported from Greece (Vondrak & al. 2012) and Italy (Ravera & Brunialti 2013).

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Veloporphyrellus latisporus, a new generic record for India

Faisal Mushtaq[1], Wasim Sajad Malik[2], Dyutiparna Chakraborty[3], Musadiq Hussain Bhat[4], Ashish Vyas[*1], Kanad Das[5*]

[1] *Central Department of Botany, Lovely Professional University*
    Punjab144001, India
[2] *Department of Botany, section of Mycology and Plant Pathology, University of Kashmir*
    Srinagar,190006, India
[3] *Eastern Regional Center, Botanical Survey of India*
    Shillong 793003, India
[4] *Division of Fruit Science, Sher-i-Kashmir University of Agricultural Science and Technology of*
    Kashmir, Shalimar, Srinagar, Jammu & Kashmir,190025, India
[5] *Central National Herbarium, Botanical Survey of India*
    Botanic Garden, Howrah, 711103, India

* Correspondence to: ashish.vyas@lpu.co.in, daskanadbsi@gmail.com

Abstract—During routine macrofungal surveys in various temperate forests of Indian Himalayan belt, we made some collections of the interesting genus *Veloporphyrellus.* After through literature review, morphological examinations, and phylogenetic analysis of two ribosomal genes, those collections were found to be conspecific with *Veloporphyrellus latisporus,* which was described from coniferous forests of Pakistan. We present the genus and the species from India for the first time with detailed morphological description, illustrations, multigene phylogenetic analysis, and comparisons with related species.

Key words—*Austroboletoideae, Boletaceae,* Himachal Pradesh, Jammu & Kashmir, taxonomy

Introduction

Veloporphyrellus L.D. Gómez & Singer (*Boletaceae*), was typified by *V. pantoleucus* L.D. Gómez & Singer, described from Costa Rica (Gómez & Singer 1984). Subsequently, nine other *Veloporphyrellus* species were described, from Asia, Africa, and North America (http://www.indexfungorum.org/

names/names.asp). Combination of characters like pinkish or greyish pink hymenophore, pileus margin with a membranous veil, smooth basidiospores and trichodermous nature of pileipellis make this genus morphologically distinct within *Boletaceae*. Moreover, multigene phylogenetic analysis placed *Veloporphyrellus* species within the subfamily *Austroboletoideae* (Wu & al. 2014), although the genus is polyphyletic (Li & al. 2014, Wu & al. 2016, Gelardi & al. 2021, Ayala-Vásquez & al. 2022).

Being a megadiverse country, India exhibits a potential macrofungal diversity along with its floral and faunal diversity. Members of an ectomycorrhizal family like *Boletaceae* are explored time to time from different part of Himalayas (Berkeley 1852, Lakhanpal 1996, Sharma & al. 2005, Das 2009, 2012, 2013; Kour & al. 2013, Chakraborty & Das 2015, Das & al. 2014, Chakraborty & al. 2018). During routine macrofungal surveys to the different parts of Western Himalayan region ranging from Jammu & Kashmir (Kishtwar district; Ramban district) to Himachal Pradesh (Chamba district) authors came across a few interesting members of *Boletaceae*. After detail macro- and micromorphological characterization combined with phylogenetic studies based on nrITS and nrLSU sequences revealed our present collections to be *Veloporphyrellus latisporus*, which was originally described from Pakistan (Khan & al. 2021).

Here we report the genus *Veloporphyrellus* as well as the species *V. latisporus* for the first time from India, based on a detailed morphological description, illustration, and multi-gene phylogenetic inference.

Materials & methods

Morphological analysis

Young to mature basidiomata were collected during surveys in forested areas of Jammu & Kashmir and Kalatop Wildlife Sanctuary (Himachal Pradesh) during the monsoon season, July and August, in 2021–22. Macromorphological characterizations were noted in the field or at basecamp from fresh and dissected basidiomata. Photographs were taken in the field with a Canon Power Shot SX 50 HS camera. Color codes and terms mostly follow Kornerup & Wanscher (1978). Samples were dried in a field drier. Micromorphological characters were observed after mounting freehand sections of dried materials in a solution of 5% KOH, 1% Phloxin, and 1% ammoniacal Congo red with an Olympus CX 41 compound microscope. Drawings of the micromorphological features were made with the help of drawing tube at 1000× magnification. Microscopic photographs were taken with an Olympus BX 53 camera. The basidiospores were measured in lateral view. Basidiospore measurements and length/width ratios (Q) are recorded as: minimum–mean–maximum. Basidium length excludes the length of sterigmata. The specimens are conserved in the Central National Herbarium, Botanical Survey of India, Howrah, India (CAL) and the

Herbarium, Centre of Biodiversity and Taxonomy, University of Kashmir, Srinagar, India (KASH).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 100 mg of dried basidiomata with the InstaGeneTM Matrix Genomic DNA isolation kit (Biorad, USA) following the manufacturer's instructions. The nrITS and nrLSU genes regions were amplified with primer pairs ITS-1F and ITS-4 (White & al. 1990; Gardes & Bruns 1993) and LR0R and LR5 (Vilgalys & Hester 1990), respectively. PCR amplification was performed on a thermal cycler (Eppendorf, Germany) programmed for 5 min at 95°C, 30 cycles of 1 min at 95°C, 30 s at 52°C, 2 min at 72°C, and a final 7 min extension step at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragment were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the amplifying primers. The sequence quality was checked using Sequence Scanner Software v. 1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious v. 5.1 (Drummond & al. 2010). The newly generated sequences in this study were submitted to GenBank (http://www.ncbi.nlm.nih.gov/Genbank/).

Sequence alignment and phylogenetic analysis

The newly generated nrITS and nrLSU sequences of *V. latisporus* from India and its close relatives were retrieved from the nBLAST search against GenBank (https://www.ncbi.nlm.nih.gov/genbank), and relevant published phylogenies (Li & al. 2014, Khan & al. 2021). Two datasets (ITS and LSU) were created separately. Both the datasets were aligned separately using the online version of MAFFT v. 7 with the L-INS-i strategy (Katoh & al. 2019) and default settings. To eliminate ambiguously aligned positions in the alignment as objectively as possible, Gblocks 0.91b (Talavera & Castresana 2007) was used. The program was run with settings allowing for smaller blocks, gaps within these blocks and less strict flanking positions. Each nrLSU and nrITS datasest were then phylogenetically analysed using the maximum-likelihood (ML) method. For the ML analysis, the concatenated alignment was carried out using raxmlGUI v. 2.0 (Edler & al. 2021) with the GTRGAMMA substitution model. The ML analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. Maximum-likelihood bootstrap values ≥70% are shown in both phylogenetic trees. *Tylopilus fellus* was used as the outgroup taxon for both analyses.

Phylogeny

The ITS data matrix contained 16 sequences and the alignment comprised 962 characters. The LSU matrix contained 27 sequences and the alignment comprised 1357 characters.

In our nrITS-based phylogenetic estimation (Fɪɢ. 1), sequences derived from Indian collections nested within the clade of five Pakistani *Veloporphyrellus latisporus* collections. In our nrLSU-based phylogenetic estimation (Fɪɢ. 2),
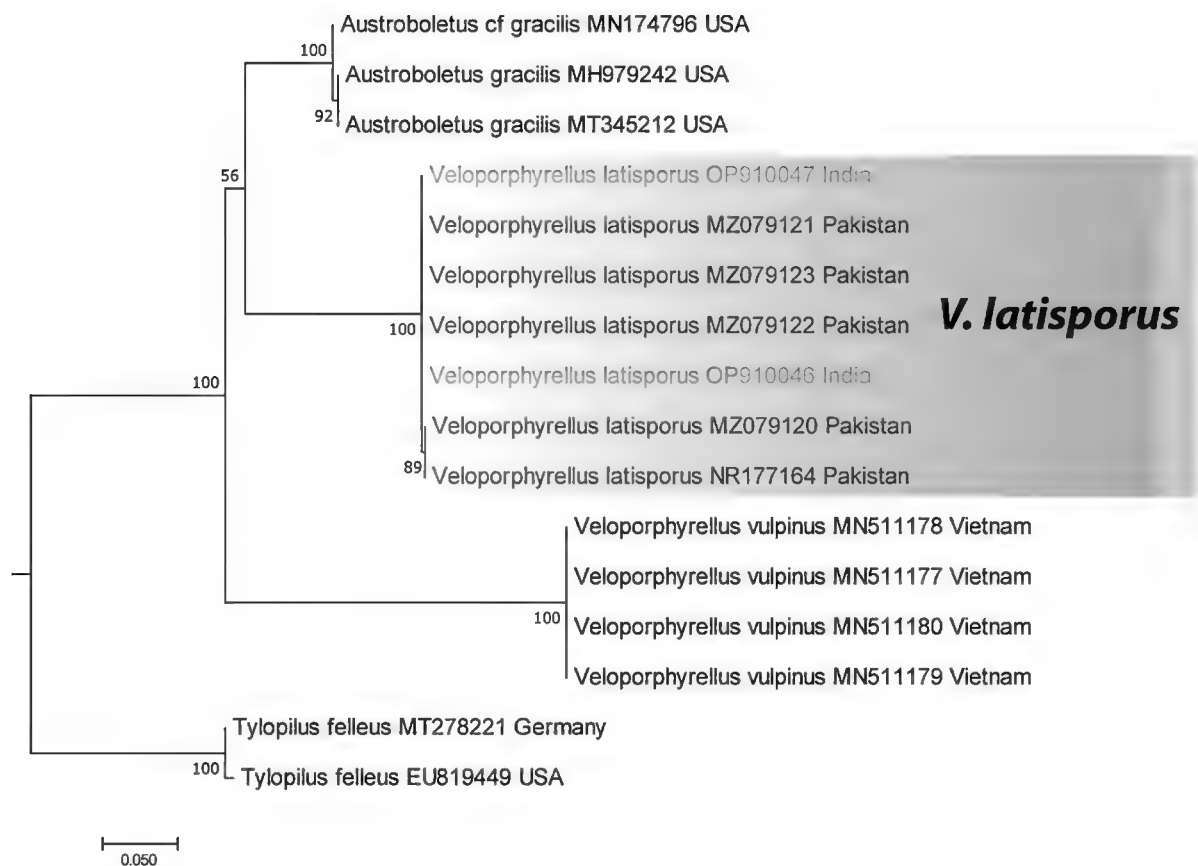
FIG 1: Maximum likelihood phylogenetic tree generated from the analysis of the nrITS sequences of *Veloporphyrellus latisporus* and closely allied species. Maximum likelihood bootstrap support values ≥70% are shown at branch-nodes.
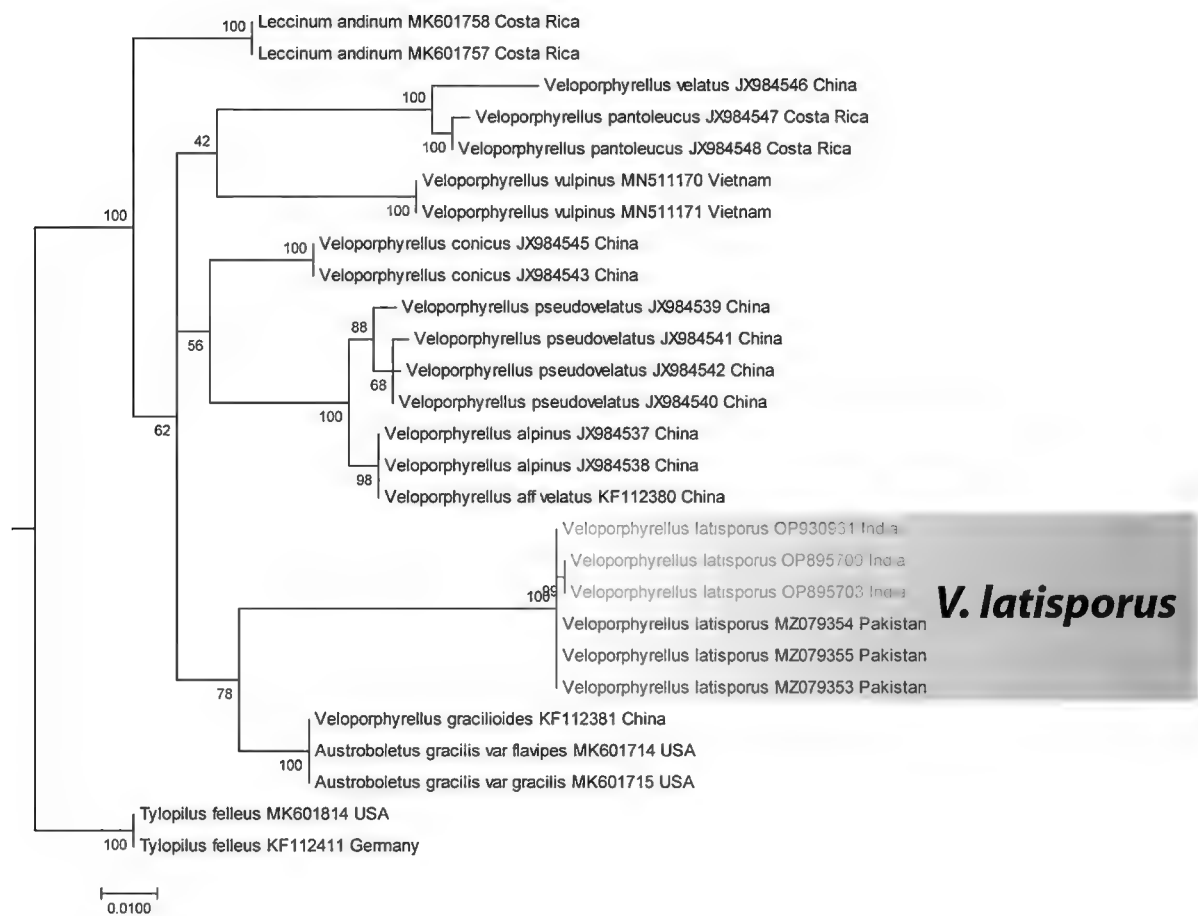

FIG 2: Maximum likelihood phylogenetic tree generated from the analysis of the nrLSU sequences of *Veloporphyrellus latisporus* and closely allied species. Maximum likelihood bootstrap support values ≥70% are shown at branch-nodes.

Veloporphyrellus is better resolved; and sequences derived from our Indian collections nested within the clade consisting of three Pakistani *V. latisporus* collections. Both phylogenetic analyses strongly suggest that out Indian collections are conspecific with the South Asian *V. latisporus*.

Taxonomy

Veloporphyrellus latisporus J. Khan & S. Ullah, Nordic J. Bot. 39(9): e03178. 2021.   Figs 3, 4
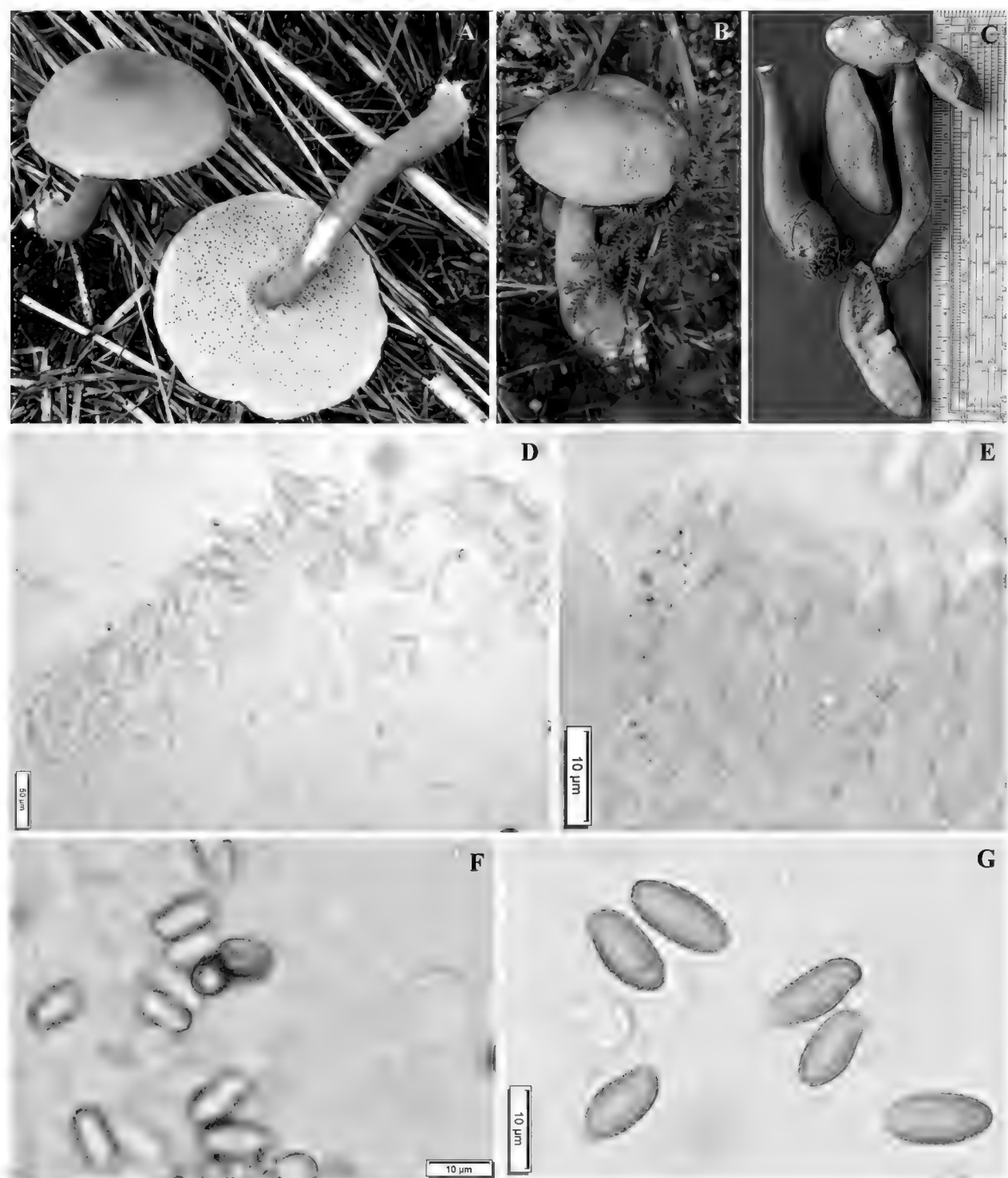

Fig 3: *Veloporphyrellus latisporus* (KASH 8619 [A]; CAL 1905 [B–G]). A–C. Basidiomata in field and in basecamp; D. Pileipellis; E. Basidia; F. Cheilocystidia; G. Basidiospores. Scale bars: D = 50 μm; E–G = 10 μm.
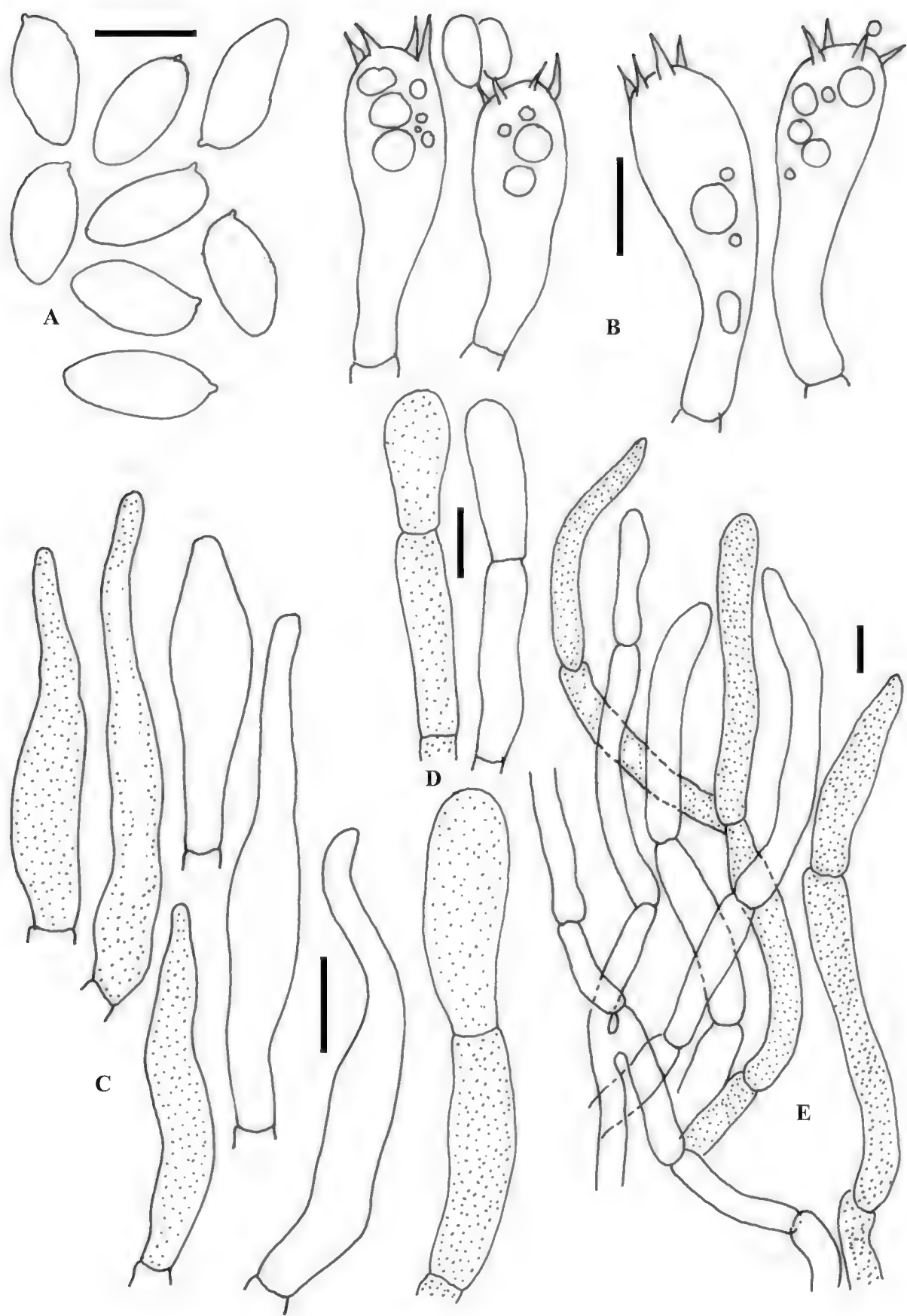
FIG 4: *Veloporphyrellus latisporus* (CAL 1905). A. Basidiospores; B. Basidia; C. Pleurocystidia; E. Cheilocystidia; F. Pileipellis. Scale bars= 10 μm.

Basidiomata epigeous, medium-sized. Pileus 50–78 mm broad, convex, plano-convex with age; margin plane, velar remnants present as a sterile flap of tissues, entire, sometimes cracked; surface dry, smooth at first, then becoming rough to areolate and sub-tomentose to squamulose, cracked with maturity, reddish brown (8D–E4–7) to brownish red (10D4–7). Pileus context up to 47 mm thick at the disc, thinning towards the margin, pale yellow (3A2), unchanging when cut or bruised. Tube up to 3 mm deep, adnate, pinkish white; pore angular, 1–3/mm. Stipe 60–90 × 8–10 mm, central, straight to curved, cylindrical, broader at base; surface dry, non-viscid, very finely striate, brownish red to reddish brown (8E7–8). Stipe context solid, white, unchanging when cut bruised. Basal mycelium white. Taste None.

Basidiospores 12–12.5–14 × 5.5–6–6.7 μm, (n = 40; Q = 1.97–2–2.22), boletoid, appearing smooth under light microscope, pale olivaceous when observed in KOH and H_2O. Basidia 28–38 × 10–11 μm, clavate to subclavate, thin-walled, 4-spored. Pleurocystidia 38–63 × 6–10 μm, subclavate to narrowly clavate, fusiform to narrowly ventricose, thin-walled, mostly colorless in KOH and H_2O, few are pigmented. Lamellar edge fertile with basidia and cheilocystidia. Cheilocystidia 46–84 × 8–13 μm, subclavate to subcylindrical, septate, abundant. Hymenophoral trama composed of colorless and hyaline cylindrical hyphae. Pileipellis trichodermous, composed of erect or ascending, interwoven, septate hyphal terminations; terminal elements measuring 33–54 × 7–9 μm, mainly cylindrical or sometimes narrow at the apex, apically rounded, thin walled. Caulocystidia not found. Clamp connections absent in all tissues.

Ecology & Distribution—occurrence under moist coniferous forests; known from Pakistan and India.

Specimens Examined—INDIA. Himachal Pradesh: Chamba district, Kalatop Wildlife sanctuary, alt. 2398 m, on soil under *Pinus wallichiana* A.B. Jacks., 28 Aug. 2021, D. Chakraborty DC 21-75 (CAL 1905; GenBank OP910046, OP895703); Jammu & Kashmir: Kistwar district, Bindraban, alt. 1410 m, on soil under *Pinus wallichiana* A.B. Jacks., 10 July. 2022, F. Mushtaq FM 22-0010 (CAL 1906; GenBank OP910047, OP895709); Ramban district, Gool, alt. 2200 m, on soil under *Abies pindrow* (Royle ex D. Don) Royle, 31 August 2022, W.S. Malik 41-Aug-22 (KASH 8619; GenBank OP930961).

Discussion

A combination of macro- and micromorphological characters identify our Indian collections as *Veloporphyrellus latisporus*: small to medium-sized basidiomata with areolate to granulose, reddish brown to brownish red pileus; pinkish buff hymenophore; unchanging context color; white basal mycelium;

trichodermous nature of pileipellis; occurrence under conifers like *Pinus* and *Abies*; and multigene (nrITS and nrLSU) phylogenetic analyses undoubtedly identify the present species as *V. latisporus*. In the ITS analysis, the *V. latisporus* clade is sister to a clade consisting of *Austroboletus gracilis* and *A.* cf. *gracilis* (Fɪɢ. 1). In the LSU analysis, the *V. latisporus* clade is sister to a clade consisting of *V. gracilioides, A. gracilis* var. *gracilis* and *A. gracilis* var. *flavipes* (Fɪɢ. 2). Morphologically, our Indian specimens are mostly in conformity with the *V. latisporus* holotype described from our neighboring country Pakistan (Khan & al. 2021). However, the velar remnants of the Indian specimens are present at the pileus margin as a thin sterile flap of tissue, whereas they are reported as "not observed" in the Pakistani collections.

Morphologically, *Veloporphyrellus latisporus* can be confused in the field with some other Asian species such as *V. alpinus* Yan C. Li & Zhu L. Yang, *V. pseudovelatus* Yan C. Li & Zhu L. Yang, or *V. vulpinus* T.H.G. Pham & al., but *V. alpinus* can be separated by its distribution pattern (e.g., its occurrence in higher elevations, 3100–3600 m asl) and its sharply umbonate pileus (Li & al. 2014). *Veloporphyrellus pseudovelatus* differs from our species of interest by its sub-conical, chocolate-brown pileus and prominent velar remnants at pileus margin (Li & al. 2014); and *V. vulpinus* is phylogenetically well separated from *V. latisporus* and can also be distinguished by its bitter taste (Pham & al. 2019).

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First reports of *Phylloporus gajari* from India

ANIKET GHOSH[1], MANOJ EMANUEL HEMBROM[2], DYUTIPARNA CHAKRABORTY[3], REENA GANGWAR[4], KANAD DAS[1]*

[1] *Central National Herbarium, Botanical Survey of India,*
    Botanic Garden, Howrah 711103, India
[2] *AJC Bose Indian Botanic Garden, Botanical Survey of India,*
    Botanic Garden, Howrah 711103, India
[3] *Eastern Regional Center, Botanical Survey of India,*
    Shillong 793003, India
[4] *Hemvati Nandan Bahuguna Garhwal University,*
    Srinagar, Garhwal 246174, India

*CORRESPONDENCE TO: daskanadbsi@gmail.com

ABSTRACT—During macrofungal surveys in various tropical Sal-dominated (*Shorea robusta*) forests of West Bengal, we collected specimens of *Phylloporus*. After careful literature consultation, coupled with morphological examinations and molecular phylogenetic analyses with two genes, the species was found to be conspecific with *Phylloporus gajari*, which was originally described from tropical Sal forests of Bangladesh. We report *P. gajari* as a new record from India with detailed morphological description, illustration, 2-locus phylogenetic analyses, and extensive comparisons with related species.

KEY WORDS—*Boletaceae, Boletales, Dipterocarpaceae,* macrofungi, taxonomy

Introduction

Phylloporus Quél. is a species-rich genus in *Boletaceae* and it is evident from earlier studies that tropical to subtropical regions are biodiversity hotspots for this genus (Corner 1970, 1974; Singer & Gómez 1984, Heinemann & Rammeloo 1987, Singer & al. 1990, Neves & Halling 2010, Neves & al. 2012, Zeng & al. 2013, Hosen & Li 2015, 2017). *Phylloporus* encompasses a group of species with predominantly lamellate instead of poroid hymenophore (Singer 1986; Neves

& Halling 2010; Neves & al. 2012). Approximately 95 named taxa in this genus have been reported worldwide (https://www.indexfungorum.com) excluding subspecies and varieties. Due to phenotypic plasticity, morphological species recognition in this genus is quite difficult. Neves & al. (2012) and Zeng & al. (2013) have generated molecular phylogenies studies based on multi-locus DNA sequence analyses to elaborate species delimitation and biogeographic relationships of *Phylloporus*.

Many ectomycorrhizal fungi inhabit tropical Sal-forests (*Shorea robusta*) in India (Dutta & al. 2015, Ghosh & al. 2022a,b; Hembrom & al. 2017, Khatua & al. 2015, 2017, 2021; Paloi & Acharya 2019, Verma & al. 2018), but the diversity of boletes is unfortunately poorly understood. Only few species of boletes have been reported from these forests (Parihar & al. 2014, 2018a,b; Chakraborty & al. 2022). Recently, we have collected several basidiomata of a *Phylloporus* species during our macrofungal surveys to *Shorea robusta*-dominated forests in West Bengal. Significant macro- and micromorphological features combined with phylogenetic studies based on nrITS and nrLSU sequences revealed our specimen to be *Phylloporus gajari*, which was originally described from Bangladesh.

Here, we report the first occurrence of *P. gajari* from India based on a detailed morphological description and a combined (nrITS + nrLSU) phylogenetic inference.

Materials & methods

Morphological analysis

Fresh young to mature basidiomata were collected during field surveys to various areas of West Bengal during the rainy season (July and August), in 2020–22. Macromorphological characters were recorded in the field or at basecamp from fresh and dissected fruitbodies. Photographs were taken in the field with a Canon Power Shot SX 50 HS camera. Color codes and terms mostly follow Kornerup & Wanscher (1978). Samples were dried in a field drier. Micromorphological characters were observed from free hand sections of dried materials mounted in a solution of 5% KOH, 1% Phloxin, and 1% ammoniacal Congo red with an Olympus CX 41 compound microscope. Drawings of the anatomical features were made with a drawing tube at 1000× magnification. Microscopic photographs were taken with an Olympus BX 53 camera. The basidiospores were measured in lateral view. Basidiospores were examined in Melzer's reagent and measured in side view, excluding ornamentations. These measurements are represented as: (MIN–)AV-SD–AV–AV+SD(–MAX) spore-length × (MIN–)AV-SD–AV–AV+SD(–MAX) spore-width and Q=(MIN–)AV-SD–AV–AV+SD(–MAX), in which MIN= the minimum value, MAX= the maximum value, AV = AVERAGE value of total measured collections, SD = standard deviation and

Q = corresponds to basidiospore 'length/width ratio'. Statistics of other microscopic characters are expressed as the mean ± standard deviation with extreme values in parentheses. Basidium length excludes the length of sterigmata. Scanning Electron Microscope (SEM) images of basidiospores were obtained from dry spores that were directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with silver coating to observe patterns of spore-ornamentation at different magnifications in high vacuum mode (20 KV). SEM study was carried out with a Zeiss Evo 18 special edition model imported from Germany and installed at USIC Dept., Hemvati Nandan Bahuguna Garhwal University (HNBGU) Srinagar (Garhwal) India. The specimens are conserved in the Central National Herbarium, Botanical Survey of India, Howrah, India (CAL).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 100 mg of dried basidiomata with the InstaGeneTM Matrix Genomic DNA isolation kit (Biorad, USA) following the manufacturer's instructions. Two nuclear markers were amplified: (1) the internal transcribed spacer region of ribosomal DNA (ITS), comprising the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S, using primers ITS1-F and ITS4 (White & al. 1990; Gardes & Bruns 1993); (2) a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Vilgalys & Hester 1990). PCR amplification was performed on a thermal cycler (Eppendorf, Germany) programmed for 5 min at 95°C, 30 cycles of 1 min at 95°C, 30 s at 52°C, 2 min at 72°C, and a final 7 min extension step at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragment were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the amplifying primers. The sequence quality was checked using Sequence Scanner Software v. 1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious v. 5.1 (Drummond & al. 2010). The newly generated sequences in this study were submitted to GenBank (http://www.ncbi.nlm.nih.gov/Genbank/). Accession numbers of species used in the phylogenetic analyses are listed in Table 1.

Sequence alignment and phylogenetic analysis

The newly generated nrITS and nrLSU sequences of *Phylloporus gajari* (CAL 1903 and CAL 1904) from India and their close relatives were retrieved from the nBLAST searches against GenBank (https://www.ncbi.nlm.nih.gov/genbank), and relevant published phylogenies (Hosen & Li 2015, 2017; Zeng & al. 2013). Two datasets (ITS and LSU) were created separately. Both the datasets were aligned separately using the online version of MAFFT v. 7 with the L-INS-i strategy (Katoh & al. 2019) and then trailing ends of the alignment trimmed manually with MEGA v. 7 (Kumar et al. 2016). To eliminate ambiguous positions in the alignment as objectively as possible, Gblocks 0.91b (Talavera & Castresana 2007) was used. The program was run with settings allowing for smaller blocks, gaps within these blocks and less strict flanking positions. Species delimitation was first examined using single locus phylogenies.

TABLE 1. *Phylloporus* and *Xerocomus* species and sequences used in the phylogenetic analyses. New sequences are set in bold font.

SPECIES	VOUCHER	COUNTRY	GenBank accession no.	
			ITS	LSU
P. alborufus	MAN022	Costa Rica	JQ003624	JQ00367
P. attenuatus	HKAS 76167	Bangladesh	KR094776	KR094780
	HKAS 76168	Bangladesh	KR094777	KR094781
P. bellus	HKAS 42850	China	JQ967240	JQ967197
P. bogoriensis	DED7785	Indonesia	JQ003625	JQ003680
P. brunneiceps	HKAS 59551	China	JQ967242	JQ967199
P. caballeroi	REH7906	Panama	JQ003638	JQ003662
P. castanopsidis	MAN104	Thailand	JQ003642	JQ003689
P. catenulatus	HKAS 76156	Bangladesh	KR094774	KR094778
	HKAS 76157	Bangladesh	KR094775	KR094779
P. cyanescens	REH8681	Australia	JQ003621	JQ003684
P. dimorphus	MAN128	Thailand	JQ003648	JQ003697
P. foliiporus	JLM1677	USA	JQ003641	JQ003687
P. gajari	**AG 20-003**	**India**	**OP550185**	**OP550198**
	AG 22-001	**India**	**OP550186**	**OP550196**
	HKAS 76158	Bangladesh	KR231696	KR231697
	HKAS 76159	China	KP780416	KP780420
	HKAS 76161	Bangladesh	KP780417	KP780421
	HKAS 76166	Bangladesh	KP780418	KP780422
	HKAS 81585	Bangladesh	KP780419	KP780423
	OR1104	Thailand	MT880254	—
	PHY	Thailand	MN580130	—
P. imbricatus	HKAS 54647	China	JQ967245	JQ967202
P. leucomycelinus	MB00-43	Panama	JQ003628	JQ003677
P. orientalis	REH8755	Australia	JQ003651	JQ003701

SPECIES	VOUCHER	COUNTRY	GenBank accession no.	
			ITS	LSU
P. pachycystidiatus	HKAS 54540	China	JQ967254	JQ967211
P. pelletieri	Q7199c	Slovakia	JQ003639	JQ003668
P. rhodoxanthus	JLM1808	USA	JQ003654	JQ003688
P. rubeolus	HKAS 52573	China	JQ967259	JQ967216
P. rubiginosus	MAN117	Thailand	JQ003645	JQ003692
P. rubrosquamosus	HKAS 54542	China	JQ967260	JQ967217
P. rufescens	HKAS 59722	China	JQ967263	JQ967220
P. scabripes	REH8531	Belize	JQ003623	JQ003683
P. species	HKAS 74679	China	JQ967271	JQ967228
	HKAS 74680	China	JQ967272	JQ967229
	HKAS 74682	China	JQ967273	JQ967230
	HKAS 74684	China	JQ967275	JQ967232
	HKAS 74685	China	JQ967276	JQ967233
	HKAS 74687	China	JQ967278	JQ967235
	HKAS 74688	China	JQ967279	JQ967236
	HKAS 74689	China	JQ967280	JQ967237
	REH8729	Australia	JQ003650	JQ003699
P. yunnanensis	HKAS 52225	China	JQ967265	JQ967222
X. magniporus	HKAS 59820	China	JQ678697	JQ678699
X. subtomentosus	K 167686	England	JQ967281	JQ967238

When significant conflict was not observed among the single locus phylogenies, we concatenated all single locus alignments into one multi-locus dataset using BioEdit v. 7.2 (Hall 1999). The concatenated dataset was then phylogenetically analyzed using the maximum likelihood (ML) and Bayesian inference (BI) methods. For ML analysis, the concatenated alignment was carried out using raxmlGUI v. 2.0 (Edler & al. 2021) with GTRGAMMA substitution model. ML analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. For BI, the combined dataset was divided into two partitions: ITS and LSU. PartitionFinder2
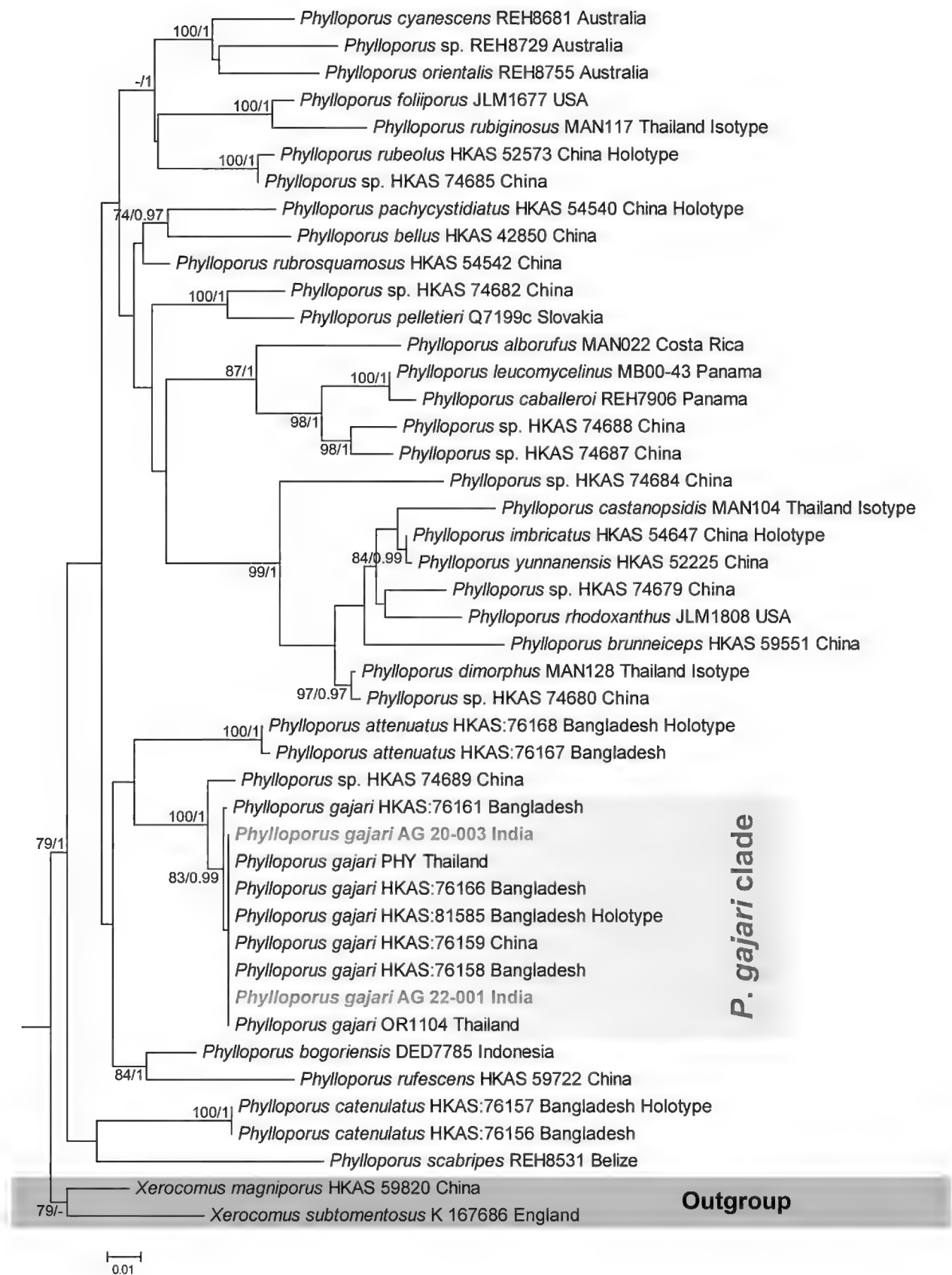
Fig. 1. Phylogram generated by Maximum likelihood analysis based on combined sequence data of nrITS and nrLSU for *Phylloporus gajari* and allied species. Maximum likelihood bootstrap support ≥70% are shown on the left of "/" and Bayesian posterior probabilities ≥0.95 are shown on the right, above or below the branches at nodes.

was used to find the substitution models for each partition (SYM+I+G for ITS, and GTR+I+G for 28S) using the Akaike information criterion (AICc) with a greedy search over all models (Lanfear & al. 2017). BI was computed in MrBayes v. 3.2.6 (Ronquist & al. 2012) with four Markov chain Monte Carlo (MCMC) chains for 1,000,000 iterations until the standard deviation of split frequencies reached below the 0.01 threshold. Trees were sampled every 100th generation. The first 25% of trees were discarded as burn-in. Chain convergence was determined using Tracer 1.5 (http://tree.bio.ed.ac. uk/software/tracer/) to ensure sufficiently large effective sample size (ESS) values (>200). Gaps in the alignment were treated as missing data in phylogenetic analyses. Maximum-likelihood bootstrap (MLbs) ≥70% and Bayesian posterior-probabilities (BPP) ≥0.95 are shown in the phylogenetic tree. *Xerocomus subtomentosus* (L.) Quél. and *X. magniporus* M. Zang & R.H. Petersen were used as the outgroup taxa for phylogenetic analyses.

Phylogeny

Both ML and BI analyses produced the same topology; therefore, only the maximum likelihood tree with both MLbs and BPP values are shown (Fig. 1). The nrITS data matrix contained 45 sequences and the alignment comprised 429 characters. The nrLSU data matrix contained 43 sequences and the alignment comprised 852 characters. The final combined dataset (nrITS + nrLSU) contained 45 sequences including our consensus sequences. The final alignment comprised 1281 characters including gaps.

Our combined 2-locus phylogenetic analyses show that sequences derived from our Indian collections nested (with robust support; MLbs = 83%, BPP = 0.99) within the clade consisting of seven *P. gajari* collections from Bangladesh, China, and Thailand. Our phylogenetic analyses strongly suggest that out Indian collections are conspecific with Asian *P. gajari*.

Taxonomy

Phylloporus gajari Hosen & Zhu L. Yang, Mycoscience 56(5): 585. 2015   Figs 2, 3

Basidiomata epigeous, small to medium-sized. Pileus 22–60 mm broad, convex to plano-convex when young, becoming applanate, depressed to broadly depressed at center and infundibuliform with age; margin plane to uplifted with age, entire; surface dry, smooth at first, then becoming rough to areolate and subtomentose to squamulose, reddish brown (8D–E4–7) to brownish red (10D4–7) when young becoming pale yellow (3A4), yellowish brown to pale yellow-brown (5D–E5–7) at maturity. Pileus context up to 7 mm thick at center, thinning towards the margin, pale yellow to yellowish white (3A2–3), turning greenish blue (24A–C6–8) when cut or bruised. Lamellae up to 5 mm
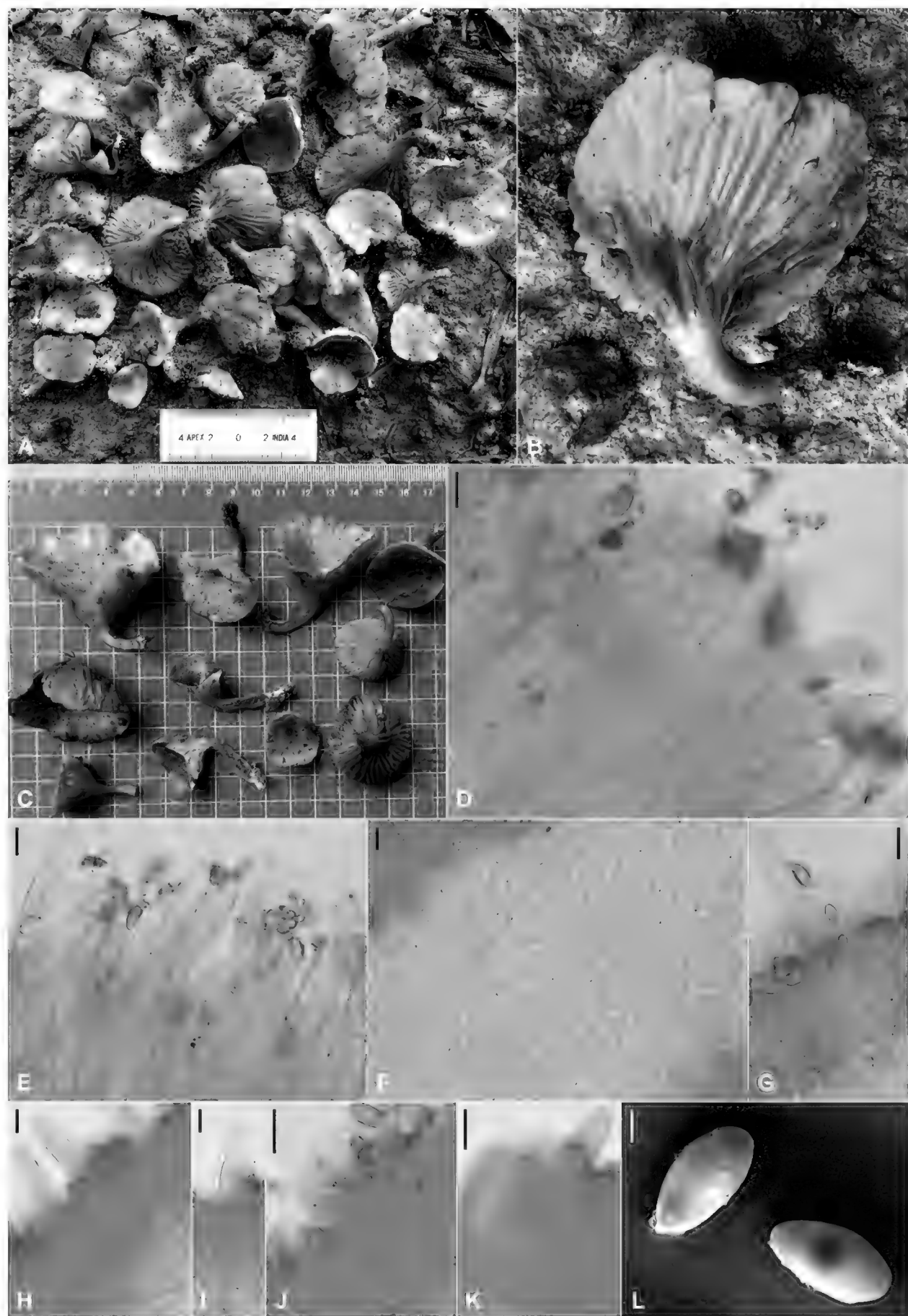
FIG. 2. *Phylloporus gajari* (CAL 1903; CAL 1904). A–C. Fresh and dissected basidiomes in the field and basecamp; D, E. Transverse section through pileipellis; F. Hymenophoral trama; G–I. Pleurocystidia; J. Basidia; K. Cheilocystidia; L. SEM image of basidiospores. Scale bars: D, E = 10 μm; F = 20 μm; G–K = 10 μm; L = 2 μm.
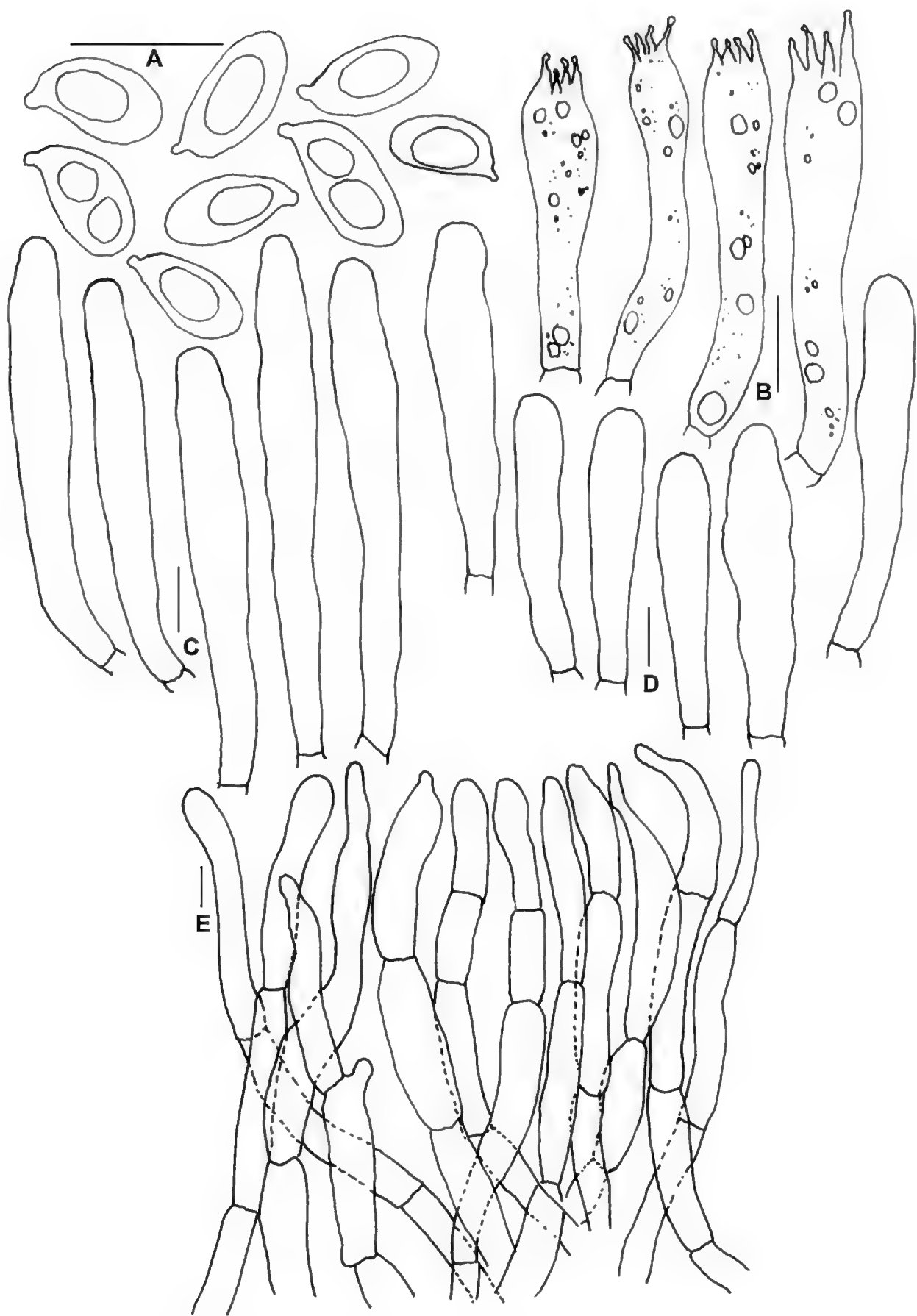
FIG. 3. *Phylloporus gajari* (CAL 1903; CAL 1904). A. Basidiospores; B. Basidia; C. Pleurocystidia; D. Cheilocystidia; E. Pileipellis. Scale bars: A−E = 10 μm.

deep, distant to subdistant (4–6/cm at pileus margin), pastel yellow, light yellow to yellow (2–3A4–7) with reddish brown spots, moderately thick, turning greenish blue (24A–C6–8) when cut or bruised; forked near stipe apex, midway to the margin, or near the margin; lamellulae present in different lengths; edges entire and concolorous. STIPE 20–55 × 5–8 mm, central, straight to curved, cylindrical; surface dry, very fine squamulose, brownish red to reddish brown (9C–E6–8). STIPE CONTEXT solid, becoming stuffed with age, pale yellow (3A2–3); turning blue slowly in the upper part but usually unchanging in the lower half on exposure. BASAL MYCELIUM white.

BASIDIOSPORES (7.0–)8.1–9.1–10.2(–11.0) × (3.5–)3.9–4.2–4.6(–5.0) μm, Q = (1.67–)1.9–2.16–2.41(–2.63), subcylindrical, wall up to 0.5 μm thick, appearing smooth under light microscope but with bacillate ornamentation under scanning electron-microscope (SEM), inamyloid, pale yellow to yellow when observed in KOH and H_2O. BASIDIA 25–45 × 6–9 μm, cylindrical to subclavate, thin-walled, mostly 4-spored, sterigmata up to 6 μm long. PLEUROCYSTIDIA 62–90 × 8.5–13 μm, subclavate to narrowly clavate with obtuse-rounded apex, thin-walled, colorless in KOH and H_2O. LAMELLAR EDGE fertile with basidia and cheilocystidia. CHEILOCYSTIDIA 46–64 × 8–12 μm, with the same shape as pleurocystidia. HYMENOPHORAL TRAMA *phylloporus*-type. PILEIPELLIS palisadoderm, composed of parallel to subparallel hyphal cells; terminal elements thin-walled, composed of chains of 4–5 cells, sometimes branched, 31–61 × 5–11 μm, mainly cylindrical or sometimes narrow at the apex, apically obtuse-rounded, colorless in KOH and H_2O. CAULOCYSTIDIA 24–45 × 8–14 μm, ventricose to subclavate with obtuse-rounded apex, thin-walled, colorless in KOH and H_2O. CLAMP-CONNECTIONS absent in all tissues.

ECOLOGY & DISTRIBUTION—occurring under *Shorea robusta* in tropical deciduous forests; known from India, Bangladesh, China, and Thailand.

SPECIMENS EXAMINED—**INDIA. WEST BENGAL: Bankura district**, Joypur forest, alt. 73 m, on soil under *Shorea robusta* Roth (*Dipterocarpaceae*), 8 Jul 2020, A. Ghosh & M.E. Hembrom AG 20-003 (CAL 1903; GenBank OP550185, OP550198); **Jhargram district**, Lodhasuli, alt. 80 m, on soil under *S. robusta*, 14 Aug 2022, A. Ghosh AG 22-001 (CAL 1904; GenBank OP550186, OP550196).

Discussion

A combination of macro- and micromorphological characters such as small to medium-sized basidiomata with areolate to sub-tomentose, reddish brown to brownish red pileus, yellowish decurrent lamellate hymenophore staining greenish blue when cut or bruised, white basal mycelium, palisadoderm structure of pileipellis and stipitipellis, occurrence under *Shorea robusta* and

2-locus (nrITS and nrLSU) phylogenetic analyses undoubtedly confirm the Indian collections as conspecific to *Phylloporus gajari*. Morphologically, the Indian specimens are mostly corresponding to the holotype described from the neighboring Bangladesh (Hosen & Li 2015). However, the Indian specimens have comparatively smaller basidiospores (cf. 9–11 × 4–5 µm; Hosen & Li 2015) and show some branched hyphae in the pileipellis.

It is quite possible that *Phylloporus gajari* could be confused with *P. attenuatus* Hosen and *P. catenulatus* Hosen & T.H. Li in the field as they share similarly sized and colored basidiomata. However, *P. attenuatus* is distinguished from *P. gajari* by more crowded lamellae and attenuated stipe with pale red to yellowish brown surface (Hosen & Li 2017); and *P. catenulatus* differs from *P. gajari* in showing small basidiomata (20–40 mm diam), and subepithelium pileipellis composed of short chains of 3–4 inflated cells (Hosen & Li 2017).

Considering the size of basidiomata and stipe color, *P. gajari* can be confused with *Phylloporus rubeolus* N.K. Zeng & al. and *P. rubiginosus* M.A. Neves & Halling. However, *P. rubeolus* has an unchanging context when cut or injured, and an ectomycorrhizal association with *Lithocarpus* spp. (Zeng & al. 2013, Hosen & Li 2015). *Phylloporus rubiginosus*, originally described from northern Thailand, differs from *P. gajari* by having yellow basal mycelium, thick-walled hymenial cystidia, encrusted clavate or sinuous caulocystidia (Hosen & Li 2015). Another species, *P. bellus* (Massee) Corner, originally described from Singapore, macroscopically is closely related to *P. gajari* but has unchangeable to slightly cyanescent lamellae on bruising, pallid yellow stipe, and comparatively shorter and wider basidiospores (8.5–10 × 4.5–5.5 µm; Corner 1971).

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MYCOTAXON

New species and records of *Endophragmiella* from freshwater and terrestrial habitats in Thailand

CHARUWAN CHUASEEHARONNACHAI[1*], SAYANH SOMRITHIPOL[1], SALILAPORN NUANKAEW[1], KANTHAWUT BOONMEE[2], NAKARIN SUWANNARACH[3*], NALIN N. WIJAYAWARDENE[4,5], NATTAWUT BOONYUEN[1]

[1] *Plant Microbe Interaction Research Team, BIOTEC, National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand*

[2] *Thailand Institute of Occupational Safety and Health (Public Organization), Bangkok 10170, Thailand*

[3] *Research Center of Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*

[4] *Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Yunnan 655011, China*

[5] *Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka, Mihintale 50300, Sri Lanka*

* CORRESPONDENCE TO: *charuwan.chu@biotec.or.th, suwan.462@gmail.com*

ABSTRACT—Seven *Endophragmiella* taxa were found in Thailand, from freshwater and terrestrial habitats in various locations. Five of them are proposed as new species: *E. acutispora*, *E. filiformis*, *E. obpyriformis*, *E. sporoprolifera*, and *E. tunicata*. *Endophragmiella acutispora* is characterized by fusiform to obclavate, 2–5-septate conidia; *E. filiformis* by obclavate, appendiculate, 3–5-septate conidia; *E. obpyriformis* by its obovoid or obpyriform, bicolored conidia; *E. sporoprolifera* by obclavate and Y-shaped, appendiculate, 5–6-septate conidia; and *E. tunicata* by cylindrical or oblong, tunicate, 2-septate conidia. Two other species, *E. multiramosa* and *E. resinae,* are new records for the Thailand mycota. Detailed descriptions, illustrations, and taxonomic keys comparing the new species with morphologically similar species are provided.

KEY WORDS—Hyphomycetes, morphology, *Sordariomycetes, Sordariomycetidae*

Introduction

Endophragmiella B. Sutton was established by Sutton (1973) for *E. pallescens* B. Sutton (type) and *E. canadensis* (Ellis & Everh.) B. Sutton. Subsequently, Hughes (1979) revised *Endophragmiella* based on morphological characteristics of its conidiogenesis, described new species, and updated the species number. Keys to *Endophragmiella* species were provided by Kirk (1985), Holubová-Jechová (1986), Wu & Zhuang (2005), and Xia & al. (2016). More than 90 taxa of *Endophragmiella* are now accepted in the genus (Index Fungorum; https://www.indexfungorum.org/Names/Names.asp, accessed 20 April 2023). This genus is mainly characterized by branched or simple conidiophores, monoblastic percurrently extending conidiogenous cells, and variable conidial shape and septation, seceding rhexolytically. *Endophragmiella* species are saprobic and occur on dead plant material, in soils, and in some cases, on *Agaricomycetes*, *Lecanoromycetes*, *Leotiomycetes*, and *Sordariomycetes* as opportunist saprobes that are distributed worldwide in tropical and temperate environments (e.g., Sutton 1973; Hawksworth 1979; Hughes 1979; Kirk 1981a, 1982a, 1983, 1985; Dunn 1982; Holubová-Jechová 1986; Wu & Zhuang 2005; Brackel & Svetlana 2009; Ma & al. 2011, 2012, 2015, 2021a; Zhurbenko & al. 2015; Xia & al. 2016; Jiang & al. 2018).

Species identification in *Endophragmiella* has been problematic due to a lack of molecular data from ex-type cultures and the difficulty of growing specimens on culture media. Identification of *Endophragmiella* species is mainly based on distinct morphological characteristics, especially in conidial form, size, septation, pigmentation, apical filament of the conidia, and conidiophores (Hughes 1979, Holubová-Jechová 1986, Wu & Zhuang 2005).

During the mycological survey of freshwater and terrestrial microfungi on plant debris from Thailand, seven taxa that resemble *Endophragmiella* were isolated, but they could not be successfully cultured. Their morphological characteristics confirmed that these taxa belong to *Endophragmiella*. Two of them were identified as new records from Thailand and the other five are proposed here as new species.

Materials & methods

Collection and morphological data

The collections were made from five freshwater and three terrestrial habitats in Thailand. Naturally decaying and submerged decaying woody specimens were randomly collected and placed in plastic bags, taken to the laboratory, and prepared

according to Chuaseeharonnachai & al. (2013). Woody samples were incubated in a moist chamber at room temperature (~25–27°C) for 7–10 days. Samples were periodically examined for the presence of microfungi under a stereomicroscope (OLYMPUS SZ61; Olympus Corporation, Japan) over 2 months. Several attempts were made to isolate the fungi in pure cultures by transferring single conidia with a fine needle to plates with different media, including potato dextrose agar (PDA; BD Difco™, Becton, Dickinson and Company, USA), corn meal agar (CMA; BD Difco™), malt extract agar (MEA; BD Difco™), potato carrot agar (PCA; HiMedia, HiMedia Laboratories Pvt. Ltd., India) and water agar (WA). Plates were incubated at room temperature for up to 30 days. No viable fungi could be isolated, suggesting that the nutritional requirements were not met by any of the tested media. DNA isolation and amplification from fungal tissues of these studying fungi by using the NucleoSpin® Plant II extraction kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) was also unsuccessful due to the small amounts of fungal material and the contamination from other microbes. For morphological examination, fungal structures were mounted in a drop of water with lactophenol and examined under higher magnification using a compound microscope (OLYMPUS CX31; Olympus Corporation, Japan). Microfungi were photographed using a Nomarski differential interference contrast microscope (OLYMPUS DP70; Olympus Corporation, Japan). The types and additional specimens were deposited in the BIOTEC Bangkok Herbarium, Pathum Thani, Thailand (BBH) [https://www.nbt-microbe.org].

Results

Seven species of *Endophragmiella* were found colonizing dead plant debris. Five of them were identified as new species by morphological characterization: *E. acutispora, E. filiformis, E. obpyriformis, E. sporoprolifera,* and *E. tunicata.* The other two are described and illustrated as new records from Thailand of *E. multiramosa* and *E. resinae.*

Taxonomy

Endophragmiella acutispora Chuaseehar., Somrith. & Boonyuen, **sp. nov.**   Fig. 1
IF 558383

Differs from *Endophragmiella acuta* by having larger conidia with more septa; and from *E. selenosporellaria* by its conidia having both basal and apical cells paler than the brown central cells.

Type: Thailand, Chiang Mai Province, Mueang Chiang Mai District, a waterfall in Chiang Mai Zoo, 18.8103°N 98.9475°E, on submerged twigs of an unidentified plant, 30 August 2019, N. Boonyuen (**Holotype**, BBH 49151).

Etymology: Latin, *acutispora,* referring to the acute conidial apex.
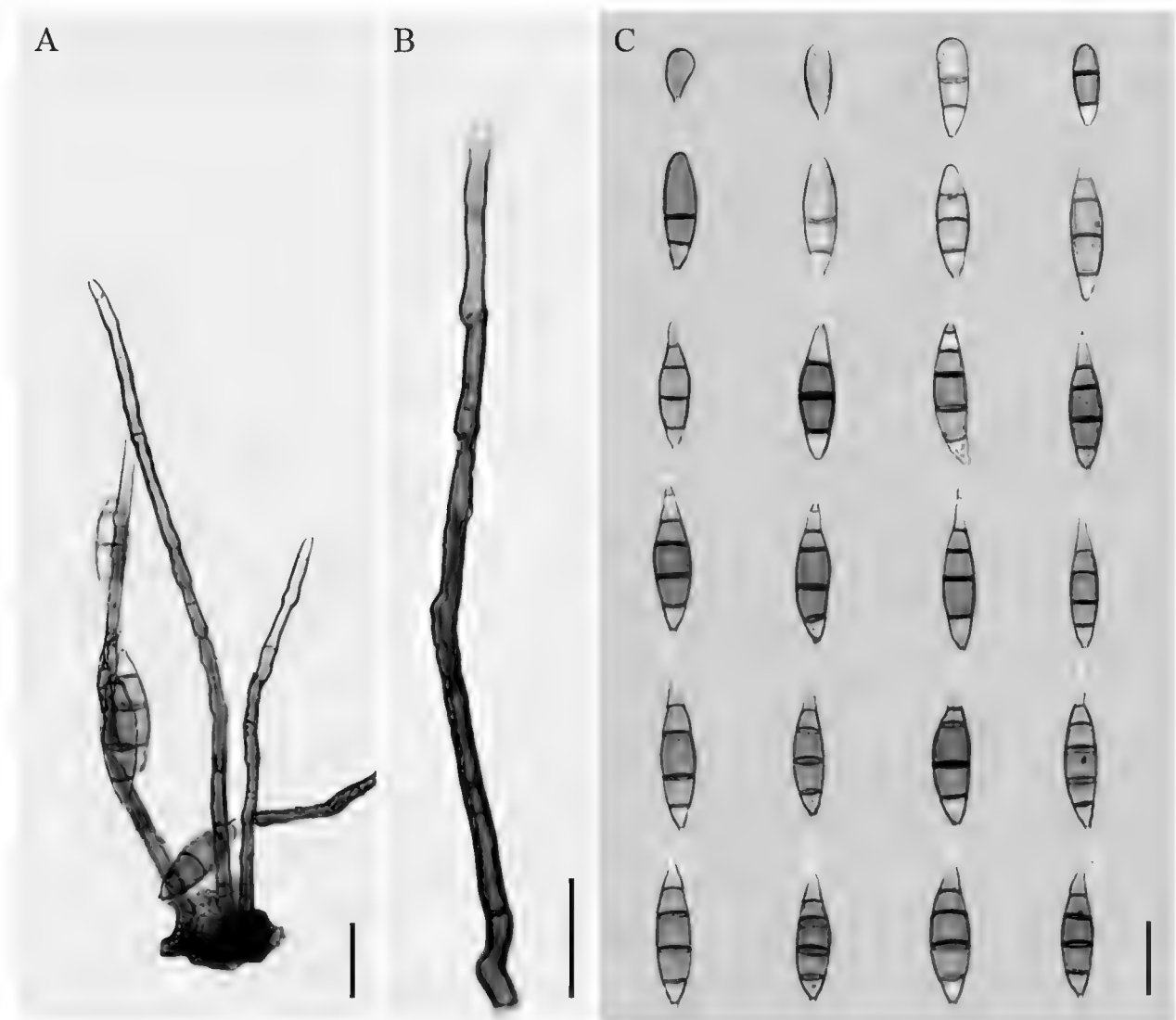
Fɪɢ. 1. *Endophragmiella acutispora* (holotype, BBH 49151): A, B. Conidiophores, conidiogenous cells and conidia; C. Conidia at different stages of development, some of which (two bottom rows) bear *Selenosporella*-like synanamorph on the apical cell. Scale bars = 25 μm.

Aꜱᴇxᴜᴀʟ ᴍᴏʀᴘʜ. Cᴏʟᴏɴɪᴇꜱ on the natural substratum effuse, hairy, brown. Mʏᴄᴇʟɪᴜᴍ partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2.5 μm diam. Cᴏɴɪᴅɪᴏᴘʜᴏʀᴇꜱ macronematous, mononematous, solitary or in small groups, cylindrical, erect, straight or flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 335 μm high, 4.5–5 μm wide at the base, with up to 10 or more percurrent extensions at the upper part. Cᴏɴɪᴅɪᴏɢᴇɴᴏᴜꜱ ᴄᴇʟʟꜱ monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. Cᴏɴɪᴅɪᴀʟ ꜱᴇᴄᴇꜱꜱɪᴏɴ rhexolytic. Cᴏɴɪᴅɪᴀ acrogenous, solitary, fusiform or obclavate, rostrate, smooth-walled, (2–)4(–5)-septate, central cells brown, basal and

apical cells paler, (42.5–)47.5–57.5 µm long, 10–12.5 µm wide at the broadest part (avg. = 50.7 × 11.7 µm, n = 50); apex acute and elongated, often bearing synanamorphic conidia; base conical and truncate with a distinct, subhyaline, 2.25–2.5 × 2.5 µm basal frill.

SYNANAMORPH: *Selenosporella*-like. CONIDIA filiform, acerose, aseptate, hyaline, 7.5–12.5 × 1.3 µm, formed on apical cell of the conidia.

SEXUAL MORPH unknown.

ADDITIONAL SPECIMEN EXAMINED—**THAILAND, CHIANG MAI PROVINCE, Mueang Chiang Mai District**, a waterfall in Chiang Mai Zoo, 18.8103°N 98.9475°E, on submerged twigs of an unidentified plant, 29 August 2019, N. Boonyuen (BBH 49152).

COMMENTS—*Endophragmiella acutispora* resembles *E. acuta* W.P. Wu and *E. selenosporellaria* Y.R. Ma & X.G. Zhang in its fusiform to obclavate conidial shape and its *Selenosporella*-like synanamorph developed from apical cells of the conidia; however, these two species differ by their smaller conidia with fewer septa (*E. acuta*, 18–25 × 8–10 µm, 3 septa, Wu & Zhuang 2005; *E. selenosporellaria*, 14.5–22 × 4.5–6.5 µm, 0–3 septa, Ma & al. 2015) and by apical conidial cells that are paler than the other cells. The conidial shape of *E. acutispora* is also morphologically similar to *E. liquidambaris* Jian Ma & X.G. Zhang, which differs by having an apical mucilaginous appendage and the absence of a *Selenosporella*-like synanamorph and by its smaller conidia with fewer septa (20–26.5 × 9–11.5 µm, 3 septa; Ma & al. 2012).

Key to *Endophragmiella acutispora* and similar species

1. Conidia with *Selenosporella*-like synanamorph 2
1. Conidia without *Selenosporella*-like synanamorph .... ***E. liquidambaris***
2. Conidia with central cells brown and other cells paler .... ***E. acutispora***
2. Conidia brown to dark brown with an apical cell paler 3
3. Conidia 3-septate, 18–25 × 8–10 µm .... ***E. acuta***
3. Conidia 0–3-septate, 14.5–22 × 4.5–6.5 µm .... ***E. selenosporellaria***

Endophragmiella filiformis Chuaseehar., Somrith. & Boonyuen, **sp. nov.**   FIG. 2
IF 558650

Differs from *Endophragmiella rostrata* in having a *Selenosporella*-like synanamorph.

TYPE: Thailand, Nan Province, Bo Kluea District, a stream near Huai Mafaen Nature Trail, 19.1481°N 101.1567°E, on a submerged twig of an unidentified plant, 14 January 2016, S. Sommai (**Holotype**, BBH 49149).

ETYMOLOGY: Latin, *filiformis*, referring to the filiform shape of the conidial appendage.
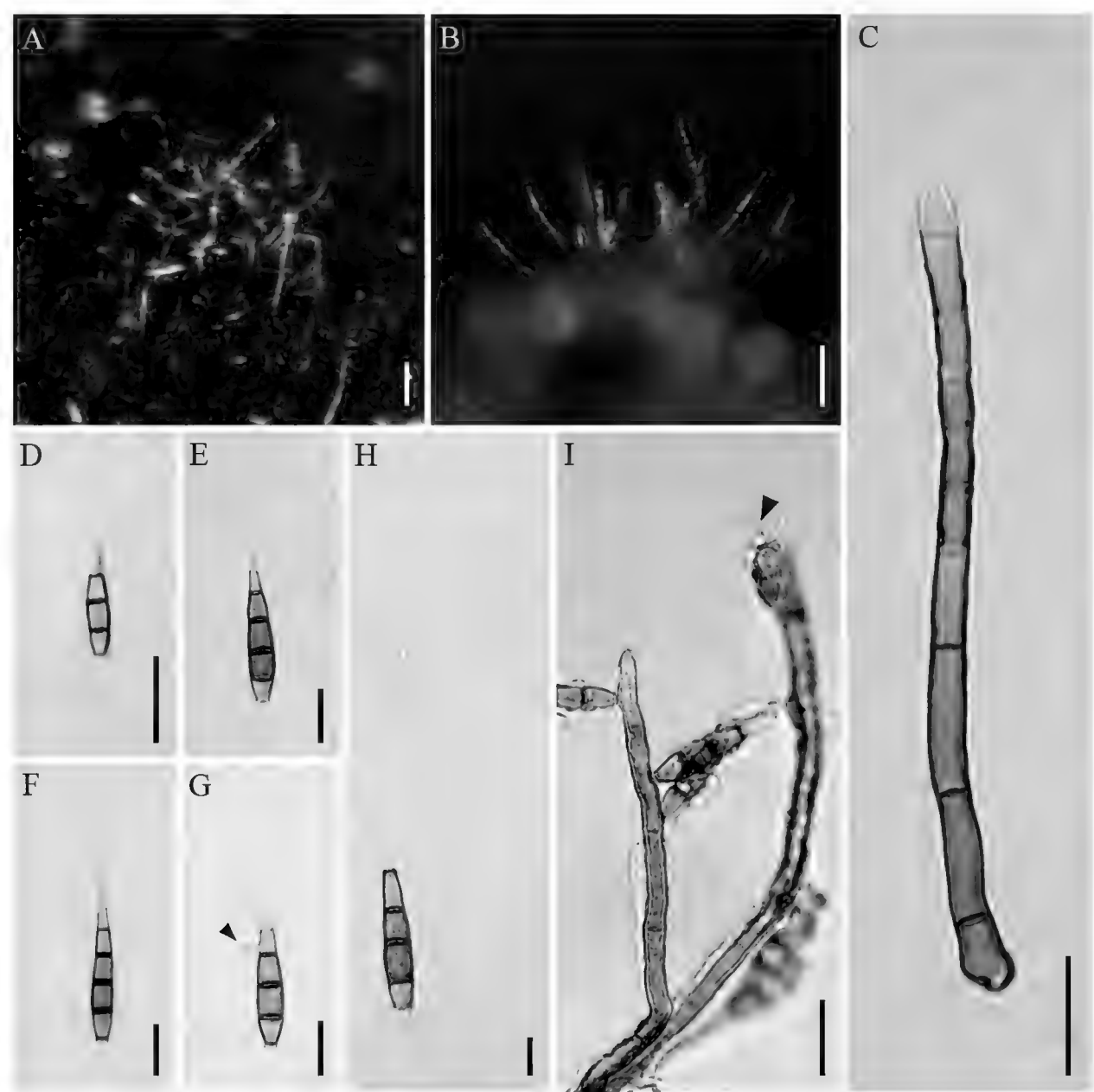
Fig. 2. *Endophragmiella filiformis* (holotype, BBH 49149): A, B. Colonies on woody substrate; C. Conidiophore and conidiogenous cell showing successive percurrent extensions; D–F, H. Conidia; G, I. Conidia with lageniform conidiogenous cells on the lateral side (arrows) bearing *Selenosporella*-like synanamorph. Scale bars: A–B = 50 μm; C–I = 10 μm.

Asexual morph. Colonies on the natural substratum effuse, hairy, brown. Mycelium partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2–2.5 μm diam. Conidiophores macronematous, mononematous, solitary, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 157.5 μm high, 2.5–5 μm wide at the base, with up to 9 or more percurrent extensions at the upper part. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. Conidial secession

rhexolytic. CONIDIA acrogenous, solitary, obclavate, rostrate, smooth-walled, (3–)4(–5)-septate, central cells pale brown, basal cell usually subhyaline but sometimes pale brown, apical cells subhyaline, body of conidium (25–)27.5–30(–32.5) μm long, 5 μm wide at the broadest part (avg. = 28.3 × 5 μm, n = 25); apex acute with a filiform, aseptate, hyaline appendage up to 10–167.5 μm long; base truncate with a distinct, subhyaline, 0.6–1.2 × 2.4–3.3 μm basal frill.

SYNANAMORPH: *Selenosporella*-like. CONIDIA filiform, acerose, aseptate, hyaline, 5–6 × 0.3–0.5 μm, developed from ampulliform or lageniform conidiogenous cells on the lateral side of the conidia.

SEXUAL MORPH unknown.

COMMENTS—The taxonomic group of *Endophragmiella* species producing rostrate conidia with a long filiform apical appendage includes *E. filiformis*, together with *E. sporoprolifera* (described below; on p. 991), *E. fusiformis* W.P. Wu, *E. nanlingensis* S.C. Ren & X.G. Zhang, *E. pulchra* (B. Sutton & Hodges) P.M. Kirk, *E. rostrata* P.M. Kirk, *E. unisetulata* (Matsush.) S. Hughes, and *E. variabilis* R.F. Castañeda. The main characteristics of *Endophragmiella* species with apical filiform appendages are summarized in TABLE 1.

Apart from possessing the apical filiform appendage, both *E. sporoprolifera* and *E. variabilis* are similar to *E. filiformis* in their conidial shape and pigmentation. However, those two species differ from *E. filiformis* by having Y-shaped conidia. Moreover, conidia of *E. sporoprolifera* are larger and lacking synanamorph (45–67.5 × 10–12.5 μm) whereas conidia of *E. variabilis* are smaller (15–23 × 4–5 μm; Castañeda-Ruíz 1988). *Endophragmiella filiformis* differs from *E. fusiformis*, *E. nanlingensis*, *E. pulchra* and *E. unisetulata* in having shorter and narrower conidia (Matsushima 1975, Hughes 1979, Kirk 1982b, Wu & Zhuang 2005, Ren & al. 2011). *Endophragmiella rostrata* was originally described by Kirk (1985). Ren & al. (2011) reported a collection of *E. rostrata* from dead branches in China, with shorter, more septate conidia than the type material. The holotype of *E. rostrata* differs from *E. filiformis* in processing its conidia with fewer septa (2–3), different pigmentation (pale brown to brown), smaller size (body of conidium 13–24 × 4–5 μm, rostrum 25–60 μm) and lacking synanamorph (Kirk 1985). In contrast, the conidial size and the number of septa of *E. filiformis* somewhat overlap with the additional collection of *E. rostrata* from Ren & al. (2011). However, *E. filiformis* can be distinguished from that collection by its conidia showing two different pigmentation patterns and having a longer appendage with the production of synanamorph.

Endophragmiella multiramosa J.L. Chen, Sydowia 60: 198 (2008)   FIG. 3

TABLE 1. Comparison of *Endophragmiella* spp. producing conidia with an apical filiform appendage. New species in bold font.

| TAXON | CONIDIAL MORPHOLOGY | | | | | | REFERENCE |
	SHAPE	SEPTATION	PIGMENTATION	BODY (µm)	APPENDAGE/ ROSTRUM (µm)	SYNANAMORPH CONIDIA (µm)	
E. filiformis	**Obclavate**	**3–5**	**Central cells pale brown, basal cell subhyaline or pale brown, apical cells subhyaline**	**25–32.5 × 5**	**10–167.5**	**Sometimes present; filiform or acerose, 5–6 × 0.25–0.5**	**This study**
E. fusiformis	Fusiform	6–7	Medium to dark brown with pale to medium brown end cells	62–80 × 7.5–9	Sometimes present (<85 × 2–2.5)	Absent	Wu & Zhuang 2005
E. nanlingensis	Broadly fusiform	3–5	Central cells brown, basal cells and apical cells pale brown	55–80 × 10–13	23–33	Absent	Ren & al. 2011
E. pulchra	Ellipsoid	3	Median cells brown, basal cell and apical cell pale brown	35–40 (including rostrum) × 5.5–6.5	8–11	Absent	Ren & al. 2011
E. rostrata	Obclavate	2–3	Pale brown to brown	13–24 × 4–5	25–60	Absent	Kirk 1985
	Obclavate	3–4	Pale brown to brown	20–30 × 4–5	25–40	Absent	Ren & al. 2011
E. sporoprolifera	**Obclavate, rarely Y-shaped**	**5–6**	**Brown; basal cells and apical cells paler**	**45–67.5 × 10–12.5**	**55–200**	**Absent**	**This study**
E. unisetulata	Fusiform	2	Central cell brown, apical and basal cells pale brown	30–36 × 9–10	24–42 × 1	Absent	Matsushima 1975, Hughes 1979
E. variabilis	Obclavate or Y-shaped	2–4	Central cells brown, upper cells and lower cells paler towards the ends	15–23 × 4–5, (branches 9–10 × 3)	28–125	Sometimes present; fusiform, 3–10 × 0.5	Castañeda-Ruíz 1988
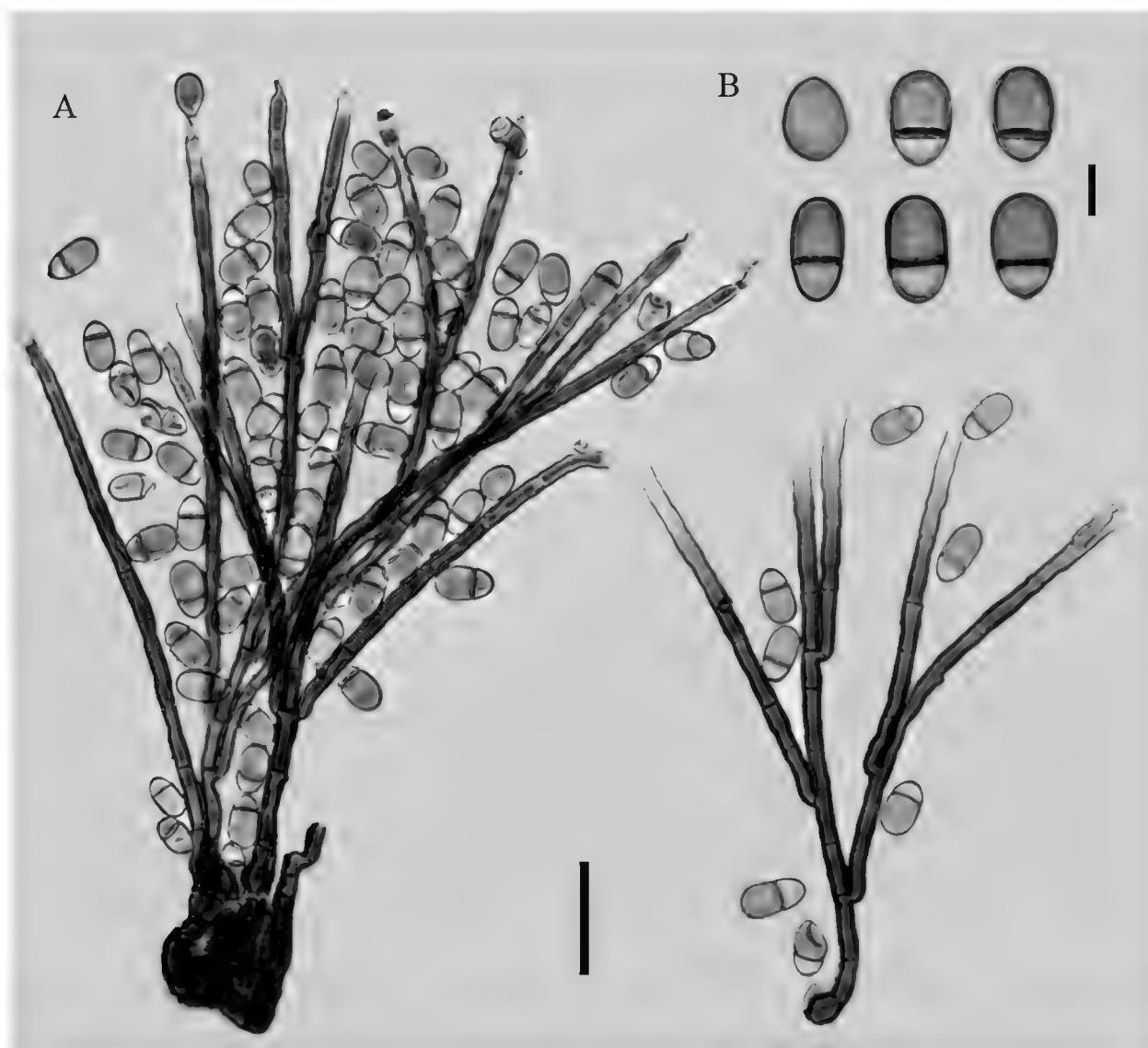
Fig. 3. *Endophragmiella multiramosa* (BBH 49148): A. Conidiophores with compactly branched stromatic base, conidiogenous cells and conidia showing inconspicuous successive percurrent extensions; B. Conidia. Scale bars: A = 20 μm; B = 5 μm.

Asexual morph. Colonies on the natural substratum effuse, tufted, brown to dark brown. Mycelium partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae. Conidiophores macronematous, mononematous, conspicuous caespitose, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, compactly branched, septate, brown, paler near the tip, up to 212.5 μm high, 2.5–6 μm wide at the base, with up to 5 or more percurrent extensions at the upper part. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. Conidial secession rhexolytic. Conidia acrogenous, solitary, obovoid, ellipsoidal, smooth-walled, usually 1-septate, basal cell pale brown, apical cell brown, 11.3–13.8 μm long, 6.3–7.5 μm wide

at the broadest part, rarely 0-septate, brown, 7.5–8 μm long, 5.8–6.3 μm wide at the broadest part, apex rounded; base truncate with a distinct, subhyaline, 0.8–1.2 × 1.2–1.8 μm basal frill.

Sexual morph unknown.

Specimen examined: **THAILAND, Bueng Kan Province, Si Wilai District**, Nature Trail, 18.1344°N 103.8814°E, on a decaying twig of an unidentified plant, 24 September 2013, C. Chuaseeharonnachai (BBH 49148).

Comments—*Endophragmiella multiramosa* was originally described by Chen & al. (2008) from a rotten twig collected in Taiwan. The new collection (BBH 49148) shares strikingly similar characteristics compared with the type species, i.e., conidial morphology, compactly branched conidiophores and a terrestrial association on dead plant material, except for the variation of the conidiophores. The conidiophores of our collection are markedly longer than those of the typical material (57.6–88 × 2.8–5.6 μm; Chen & al. 2008), although this difference is considered to reflect natural intraspecific variation. This species has not previously been recorded in Thailand; therefore, we identified this new collection as *E. multiramosa* and introduced it here as the first record from Thailand. We also consider *E. multiramosa* as a rare species, as the collection reported herein represents the only one after the original description.

Endophragmiella obpyriformis Chuaseehar., Somrith. & Boonyuen, **sp. nov.**

IF 558652   Fig. 4

Differs from *E. pentaphylacis, E. bisbyi,* and *E. suttonii* by its conidia having brown to dark brown at two upper cells.

Type: Thailand, Mukdahan Province, Mueang Mukdahan District, a small Stream in Wat Pa Tham Ta Da, 16.6125°N 104.6611°E, on a submerged decaying twig of an unidentified plant, 30 May 2019, N. Boonyuen (**Holotype**, BBH 49153).

Etymology: Latin, *obpyriformis,* referring to the obpyriform shape of the conidia.

Asexual morph. Colonies on the natural substratum effuse, hairy, brown to black. Mycelium partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2.5–5.5 μm diam. Conidiophores macronematous, mononematous, solitary or in groups of 5–7, subulate to cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 240 μm high, 5–8.8 μm wide at the base, with up to 10 or more percurrent extensions at the upper part, arising from a swollen and radially lobed basal cell. Some conidiophores are composed of a thin and pale brown cup, with an appearance resulting from a remaining wall of a

FIG. 4. *Endophragmiella obpyriformis* (holotype, BBH 49153): A. Conidiophores, conidiogenous cells and conidia; B. Conidium attached to conidiophore and bearing lateral regeneration at its basal cell (arrow); C. Conidiophore and conidiogenous cell showing successive percurrent extensions; D. Conidia. Scale bars = 20 μm.

seceded conidium. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. Conidial secession rhexolytic. Conidia acrogenous, solitary, obovoid or obpyriform, smooth-walled, (2–)3-septate, two upper cells brown to dark brown, a lower cell medium brown and a basal cell pale brown, 22–26.3(–28.8) μm long, (7.5–)10–12.5 μm wide at the broadest part (avg. = 24.5 × 10.6 μm, n = 50); apex rounded; base truncate with a distinct, subhyaline, 1.3–2.5 × 2–2.5 μm basal frill and infrequently arising from a lateral, percurrently extending conidiophore which is cylindrical, hyaline with one or more percurrent extensions.

Sexual morph unknown.

Comments—*Endophragmiella obpyriformis* belongs to the group that have obovoid or obpyriform and predominantly 3-septate conidia, including *E. bisbyi* B. Sutton ex P.M. Kirk, *E. boothii* M.B. Ellis ex P.M. Kirk, *E. clausenae* L.G. Ma & X.G. Zhang, *E. pentaphylacis* L.G. Ma & X.G. Zhang, and *E. suttonii* P.M. Kirk. However, *E. obpyriformis* differs from these species in conidial size and/or conidial pigmentation pattern. The conidia of the new species are larger than those of *E. bisbyi* (12.5–16 × 5.4–7.6 μm; Sutton 1973, Hughes 1979) but smaller than those of *E. clausenae* (31.5–42.5 × 14–16.5 μm; Ma & al. 2011) and *E. pentaphylacis* (25–32.5 × 13–16.5 μm; Ma & al. 2011). *Endophragmiella suttonii* has distinctly shorter conidia (16–18 × 9.5–11 μm; Kirk 1981b) than *E. obpyriformis*. The conidia of *E. obpyriformis* are brown to dark brown and paler toward the lower cells, while the conidia of *E. clausenae* and *E. boothii* are concolorous (Ellis 1959, Hughes 1979, Wu & Zhuang 2005, Ma & al. 2011). In addition, the conidia of *E. boothii* have thick-walled cells; the apical cell is largest and the other cells taper towards the base. (Ellis 1959, Hughes 1979, Wu & Zhuang 2005).

Moreover, *E. obpyriformis* produces conidiophores from typically radially lobed basal cells, and sometimes several conidiophores can be found with a cupped appearance.

Key to *Endophragmiella obpyriformis* and similar species
1. Conidia concolorous 2
1. Conidia versicolorous 3
2. The apical cell of conidia is largest, obtuse and thick-walled .... ***E. boothii***
2. The central cells of conidia are largest; the apical cell is acute and thin
.... ***E. clausenae***
3. Conidia obovoid to obpyriform 4
3. Conidia broadly ellipsoid to obpyriform, 25–32.5 × 13–16.5 μm .. ***E. pentaphylacis***
4. Conidia longer than 20 μm, 22–28.75 × 7.5–12.5 μm .... ***E. obpyriformis***

Endophragmiella resinae P.M. Kirk. Trans. Br. Mycol. Soc. 76: 78 (1981)   Fig. 5

Asexual morph. Colonies on the natural substratum effuse, hairy, brown to black. Mycelium partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown hyphae, 2–2.5 μm diam. Conidiophores macronematous, mononematous, solitary, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple, septate, brown, paler near the tip, up to 155 μm high, 5.5–7.5 μm wide at the base, with up to 8 or more percurrent extensions at the upper part. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown to subhyaline. Conidial secession rhexolytic. Conidia acrogenous, solitary, obovoid, obpyriform, smooth-walled, 1-septate, apical cell pale brown, basal cell paler, 15.5–21.3 μm long, 7.5–10 μm wide at the broadest part; apex rounded; base truncate with a distinct, subhyaline, 0.5 × 2.5 μm basal frill.

Sexual morph unknown.

Specimen examined: THAILAND, Nakhon Ratchasima Province, Pak Chong District, Nature Trail, 14.4323°N 101.3693°E, on a decaying twig of an unidentified plant, 9 August 2012, S. Sommai (BBH 49147).

Comments—*Endophragmiella resinae* was introduced by Kirk (1981a) and was found on an old wound of *Picea sitchensis* from Great Britain. Ren & al. (2011) reported a new collection (HSAUPH 8346) of *E. resinae* from China (on dead branches of an unidentified plant). Our collection (BBH 49147) shares similar morphological features with the type and the other representative specimens commonly found in terrestrial habitats on dead plant material (Kirk 1981a, Ren & al. 2011).

Endophragmiella resinae is closely related to a group with obpyriform, obovoid or oblong, 1-septate conidia, such as *E. boewei* J.L. Crane ex S. Hughes, *E. bogoriensis* Rifai, *E. bukkensis* Révay, *E. cambrensis* M.B. Ellis, *E. cantabrica* J. Mena & al., *E. dimorphospora* Awao & Udagawa ex P.M. Kirk, *E. multiramosa*, *E. pinicola* M.B. Ellis ex P.M. Kirk, *E. taxi* M.B. Ellis ex P.M. Kirk, *E. tuberculata* S.M. Leão & Gusmão, *E. uniseptata* M.B. Ellis ex P.M. Kirk, and *E. uniseptata* var. *pusilla* Hol.-Jech. (Ellis 1976, Hughes 1979, Holubová-Jechová 1986, Révay 1987, Chen & al. 2008, Rifai 2008, Leão-Ferreira & Gusmão 2010, Hernández-Restrepo & al. 2013).
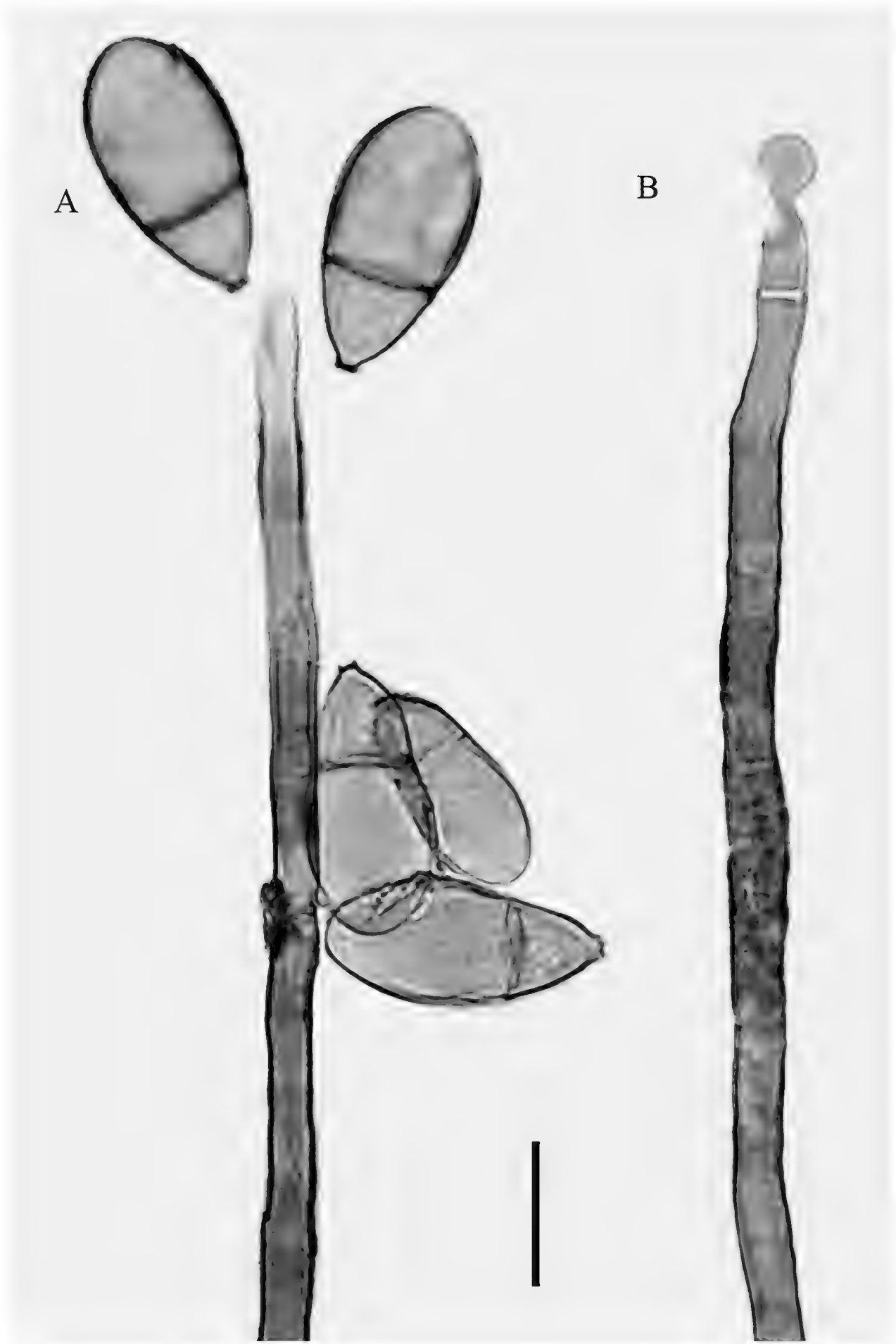
Fig. 5. *Endophragmiella resinae* (BBH 49147). A. Conidiophore, conidiogenous cell and conidia showing inconspicuous successive percurrent extensions; B. Conidiogenous cells bearing immature conidium. Scale bar = 10 μm.

It has been mentioned that *E. resinae* clearly differs from other related species by the size of the apical cell, which is typically 1.5–3 times longer than the basal cell (Ren & al. 2011). This species has been reported from Great Britain and China, and this is the first record from Thailand.

Endophragmiella sporoprolifera Chuaseehar., Somrith. & Boonyuen, **sp. nov.**
IF 558653   FIG. 6
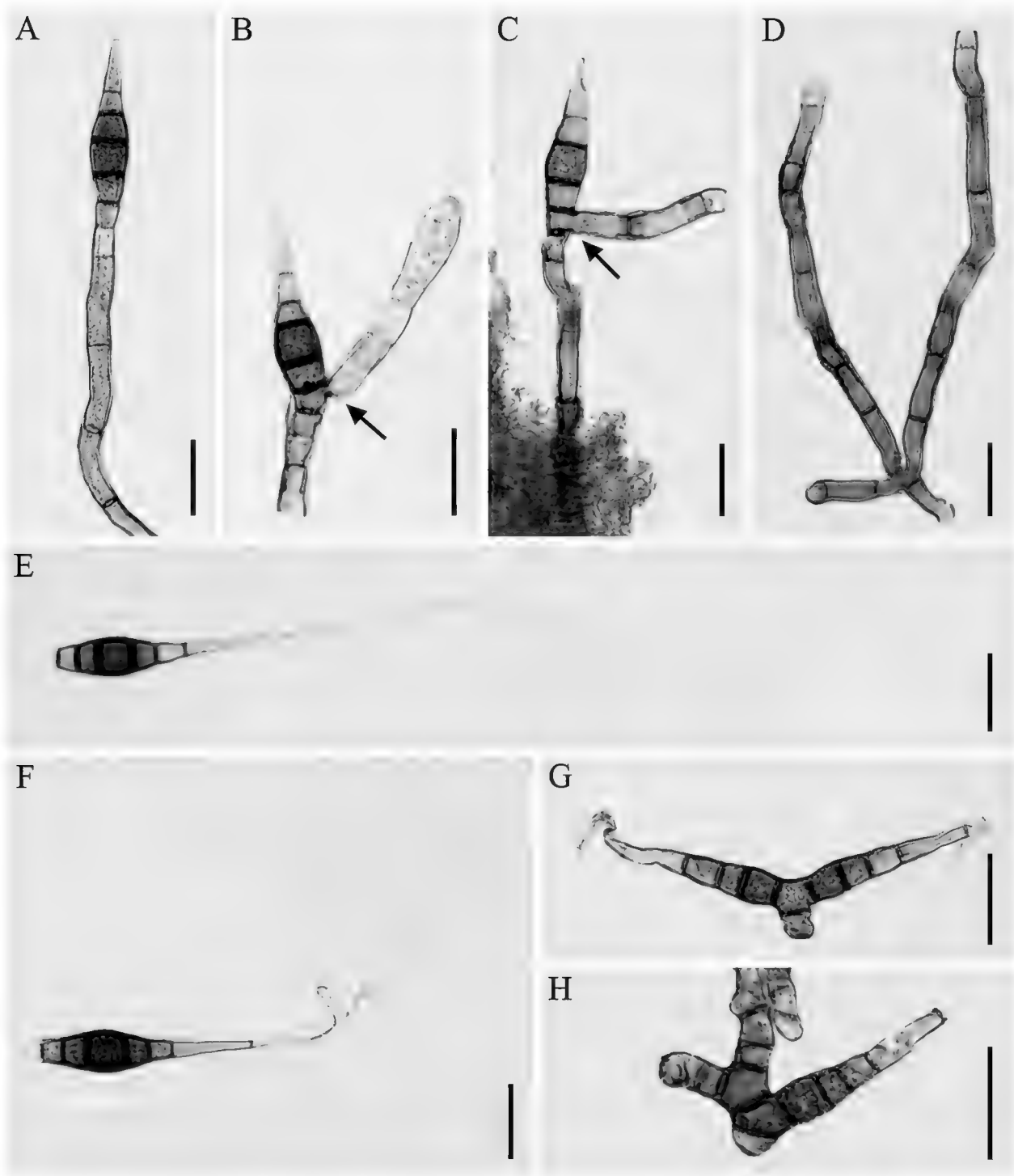
FIG. 6. *Endophragmiella sporoprolifera* (holotype, BBH 49146). A. Conidiophore and conidiogenous cell with conidium; B. Conidium attached to conidiophore and bearing lateral regeneration at its basal cell (arrow); C. Conidiophore and conidiogenous cell after successive percurrent extensions and attached conidium (arrow); D. Conidiophores and conidiogenous cells; E, F. Conidia; G, H. Y-shaped conidia. Scale bars = 20 µm.

Differs from *E. variabilis* by its larger, 5–6-septate conidia and lacking a *Selenosporella*-like synanamorph.

TYPE: Thailand, Samut Prakan Province, Phra Pradaeng District, Si Nakhon Khuean Khan Park and Botanical Garden, 13.6970°N 100.5645°E, on a decaying twig of an unidentified plant, 1 November 2014, C. Chuaseeharonnachai (**Holotype**, BBH 49146).

ETYMOLOGY: Latin, *sporoprolifera*, referring to the percurrent extensions on the conidiophore of germinated conidia.

ASEXUAL MORPH. COLONIES on the natural substratum effuse, hairy, brown. MYCELIUM partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2.5–7 μm diam. CONIDIOPHORES macronematous, mononematous, solitary, cylindrical, erect, straight or flexuous, smooth- and thick-walled, simple or occasionally branched, septate, pale brown to brown, paler near the tip, up to 182.5 μm high, 6.25–8.75 μm wide at the base, with up to 9 or more percurrent extensions at the upper part. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown to subhyaline. CONIDIAL SECESSION rhexolytic. CONIDIA acrogenous, solitary, usually obclavate, rarely Y-shaped, rostrate, smooth-walled, 5–6-septate, brown, basal cells and apical cells paler, body of conidium 45–67.5 μm long, 10–12.5 μm wide at the broadest part (avg. = 54.4 × 12 μm, n = 25); apex acute with a filiform, hyaline appendage, up to 55–200 μm long; base truncate with a distinct, subhyaline, 0.8–1.8 × 5–6.25 μm basal frill, and occasionally with a lateral percurrently extending conidiophore. The lateral conidiophore is cylindrical with up to two or more percurrent extensions.

SEXUAL MORPH unknown.

COMMENTS—*Endophragmiella sporoprolifera* is the eighth species of *Endophragmiella* with a long apical filiform appendage. *Endophragmiella sporoprolifera* is most similar to *E. variabilis* in conidial pigmentation and production of two types of conidia (obclavate and Y-shaped). However, conidia of *E. variabilis* are distinctly smaller (body of conidium 15–23 × 4–5 μm, branches 9–10 × 3 μm, rostrum 28–125 μm), possess fewer (2–4) septa, and exhibits a synanamorph (Castañeda-Ruíz 1988). *Endophragmiella sporoprolifera* somewhat resembles *E. filiformis* and *E. rostrata* in the obclavate shape of conidia but differs in having more conidial septa and larger conidial sizes (Kirk 1985). *Endophragmiella filiformis* sometimes produces synanamorph, which are not found in *E. sporoprolifera*.

Endophragmiella fusiformis, E. nanlingensis, E. pulchra, and *E. unisetulata* resemble *E. sporoprolifera* in conidial pigmentation, which is brown in central cells and pale brown in terminal cells. However, *E. sporoprolifera* differs from the others in the shape, size, and septation of conidia (Matsushima 1975, Hughes 1979, Wu & Zhuang 2005, Ren & al. 2011). *Endophragmiella sporoprolifera* is described for the first time from a decaying twig of an unidentified plant in a terrestrial habitat in an area called Bang Kachao, an island in Samut Prakan Province formed by a canal connecting the bend of the Chao Phraya River. It is influenced by high and low tides and salinity levels due to its proximity to the sea. Currently, Bang Kachao is threatened as a habit for saprobic fungi due to rapid urbanization and industrialization.

Morphologically, *Endophragmiella sporoprolifera* is remarkably similar to genus *Teratosperma* Syd. & P. Syd. in having branched conidia. However, *Teratosperma* species have conidia with a lateral branch arising from the basal cell (Ellis 1957, Wu & Zhuang 2005, Kirschner & al. 2019, Xu & al. 2021), while *E. sporoprolifera* has dimorphic conidia with obclavate and Y-shaped which branches arising from suprabasal cells. Furthermore, *E. sporoprolifera* occasionally has the production of secondary conidiophore. The secondary conidiophores formed laterally from the basal cell of the primary conidium, with two (or a few more) annellidic proliferations.

Key to *Endophragmiella filiformis* and *E. sporoprolifera* and similar species

1. Y-shaped conidia present 2
1. Y-shaped conidia absent 3
2. Conidia 5–6-septate, 100–267.5 × 10–12.5 μm (including appendage, 55–200 μm long); without *Selenosporella*-like synanamorph .... ***E. sporoprolifera***
2. Conidia, 2–4-septate, 43–148 × 4–5 μm (including rostrum, 28–125 μm long); with *Selenosporella*-like synanamorph .... ***E. variabilis***
3. Conidia concolorous, pale brown to brown .... ***E. rostrata***
3. Conidia versicolorous 4
4. *Selenosporella*-like synanamorph present .... ***E. filiformis***
4. *Selenosporella*-like synanamorph absent 5
5. Conidia ellipsoid, 35–40 × 5.5–6.5 (including rostrum, 8–11 μm long) . ***E. pulchra***
5. Conidia fusiform to broadly fusiform 6
6. Verrucose-walled conidia present .... ***E. unisetulata***
6. Verrucose-walled conidia absent 7
7. Conidia 6–7-septate, 147–165 × 7.5–9 (including appendage, up to 85 × 2–2.5 μm) .... ***E. fusiformis***
7. Conidia 3–5-septate, 78–113 × 10–13 μm (including rostrum, 23–33 μm long) .... ***E. nanlingensis***

Endophragmiella tunicata Chuaseehar., Somrith. & Boonyuen, **sp. nov.** Fig. 7

IF 558654

Differs from *E. bicolorata*, *E. bohaniensis*, *E. chinensis*, *E. collapsa*, *E. constricta*, *E. fallacia*, and *E. oblonga* by its having tunica enclosing upper cells of conidia.

Type: Thailand, Nakhon Nayok Province, a Waterfall in Pak Phli District, 14.1633°N 101.2683°E, on a submerged twig of an unidentified plant, 3 September 2014, C. Chuaseeharonnachai (**Holotype**, BBH 49145).

Etymology: Latin, *tunicata*, referring to the conidia being surrounded by a pale brown tunica.
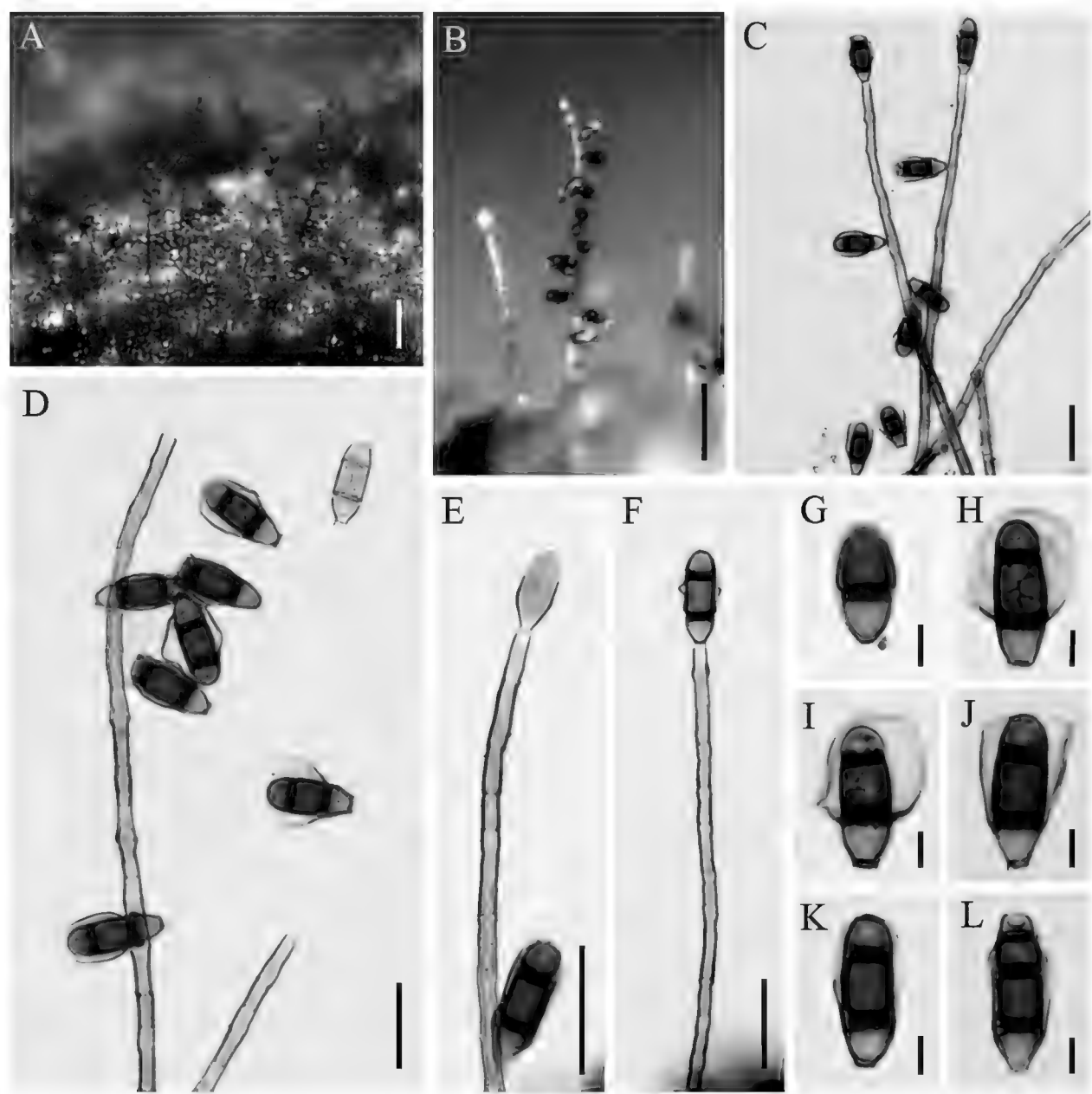
Fig. 7. *Endophragmiella tunicata* (holotype, BBH 49145). A. Colony on woody substrate; B. The seceded conidia remain attached to the lateral surface of conidiophore; C, D. Conidiophores, conidiogenous cells and conidia showing successive percurrent extensions; E. Conidiogenous cells bearing immature conidium; F. Almost mature conidium; G–L Conidia with light brown sheaths. Scale bars: A = 100 µm; B = 50 µm; C–F = 20 µm; G–L = 5 µm.

Asexual morph. Colonies on the natural substratum effuse, hairy, brown to black. Mycelium partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2–5 μm diam. Conidiophores macronematous, mononematous, solitary, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 325 μm high, 3.8–7.5 μm wide at the base, with up to 10 or more percurrent extensions at the upper part. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. Conidial secession rhexolytic. Conidia acrogenous, solitary, cylindrical or oblong, smooth-walled, (1–)2(–3)-septate, a central cell brown, basal and apical cells paler, 16.3–22.5 μm long, 6.3–8 μm wide at the broadest part (avg. = 20.4 × 7.4 μm, n = 50), surrounding by a globose to subglobose or oboviod, thin-walled, fragile, light brown, 15–20 × 7.5–12.5 μm sheath; apex rounded; base truncate with a distinct, subhyaline, 0.8–2 × 3.3–3.8 μm basal frill. A thin sheath arising from the conidial wall at the basal septum or below, usually enclosing 2 upper cells (rarely 3 upper cells) of the conidium or broken at the tip resulting in a cup shape. Sexual morph unknown.

Comments—*Endophragmiella tunicata* is considered to be in the genus *Endophragmiella* due to its appearance of rhexolytic conidial secession followed by percurrent conidiogenesis. However, *E. tunicata* is unique and differs from other *Endophragmiella* species in having a sheath enclosing the upper cells of the conidia. Therefore, this fungus is described and introduced as a new species, *E. tunicata*.

   With the exception of sheath-enclosed conidia, several *Endophragmiella* taxa have cylindrical or oblong conidia with two septa that resemble *E. tunicata*. These species are *E. bicolorata* Hern.-Restr. & al., *E. bohaniensis* N.D. Sharma, *E. chinensis* L. Qiu & al., *E. collapsa* B. Sutton ex P.M. Kirk, *E. constricta* M.T. Dunn, *E. fallacia* P.M. Kirk, and *E. oblonga* Matsush. ex S. Hughes. However, *E. tunicata* differs from *E. bicolorata*, *E. bohaniensis*, *E. fallacia*, and *E. oblonga* in conidial size. The conidia of *E. tunicata* are shorter and narrower than those of *E. bicolorata* (19–26.5 × 7.5–13 μm; Hernández-Restrepo & al. 2013), *E. bohaniensis* (18–33 × 8.5–12.5 μm; Sharma 1985), *E. fallacia* (16–30 × 9–13 μm; Kirk 1981b), and *E. oblonga* (19–28 × 9.5–11.5 μm; Matsushima 1975, Hughes 1979).

   Amongst species with overlapping ranges of conidial size, *E. tunicata* differs from *E. chinensis*, *E. collapsa*, and *E. constricta* by its conidial cell pigmentation pattern. *Endophragmiella collapsa* forms versicolorous conidia with pale brown

to dark brown upper cells and a lower cell that is paler and sometimes collapses (Sutton 1973, Hughes 1979). Conidia of *E. constricta* are versicolorous with brown upper cells and a paler lower cell, and a constricted septum is present at the median part of the conidium (Dunn 1982). Conidia of *E. chinensis* are concolorous and medium brown (Ma & al. 2021a).

Regarding habitats, the new taxon *E. tunicata* was found on woody substratum in a freshwater habitat. *E. constricta* was recorded as a secondarily opportunistic colonizer on the sclerotia of *Sclerotinia minor* Jagger, whereas other morphologically similar species were mostly saprobic species on terrestrials hosts: *E. bicolorata* on dead wood in Spain; *E. bohaniensis* on dead *Lawsonia inermis* wood in India; *E. chinensis* on dead broadleaf branches in China; *E. collapsa* on dead *Alnus* bark in Canada, *E. fallacia* on rotten *Betula pendula* in UK; and *E. oblonga* on rotten *Phyllostachys edulis* stems in Japan (Sutton 1973, Matsushima 1975, Hughes 1979, Kirk 1981b, Dunn 1982, Sharma 1985, Hernández-Restrepo & al. 2013, Ma & al. 2021a).

Key to *Endophragmiella tunicata* with similar species

1. Conidia concolorous 2
1. Conidia versicolorous 3
2. Conidiophores branched, 83–115 × 2.5–4 µm; conidia oblong or cylindrical, 11–21 × 4.5–7.5 µm .... ***E. chinensis***
2. Conidiophores unbranched, 75–300 × 5–6 µm; conidia broadly ellipsoidal, 18–33 × 8.5–12.5 µm .... ***E. bohaniensis***
3. Conidia constricted at the middle cell, 15–23 × 6.9–10.4 µm .... ***E. constricta***
3. Conidia not constricted at the middle cell 4
4. Conidia with middle cells medium brown to brown and terminal cells paler 5
4. Conidia with other pigmentation pattern 6
5. Conidia with sheath, 16.3–22.5 × 6.3–8 µm .... ***E. tunicata***
5. Conidia without sheath, 19–28 × 9.5–11.5 µm .... ***E. oblonga***
6. Conidia more variable in shape with ellipsoidal to oblong, ellipsoidal, obclavate or obovoid, 19–26.5 × 7.5–13 µm .... ***E. bicolorata***
6. Conidia less variable in shape 7
7. Conidia obpyriform to cylindrical with basal cell often collapsed, 14.4–20 × 7.2–9 µm .... ***E. collapsa***
7. Conidia broadly ellipsoid to cylindrical, 16–30 × 9–13 µm .... ***E. fallacia***

Discussion

Most *Endophragmiella* species are common in terrestrial environments. In contrast, three taxa were recorded from freshwater, including *E. bitriseptata* Goh, K.D. Hyde and *E. triseptata* C.K.M. Tsui & al. on submerged wood in

South Africa and Hong Kong, respectively, and *E. profusa* R.F. Castañeda & al. on submerged decaying *Bucida palustris* leaves in Cuba (Hyde & al. 1998, Tsui & al. 2001, Castañeda-Ruiz & al. 2010). In this study, three species were collected from terrestrial areas, while four species were reported from freshwater areas.

Of the three species described here, *E. acutispora, E. filiformis,* and *E. sporoprolifera,* they show some morphological characters that deviate somewhat from the holotype of the typical *Endophragmiella* species (*E. pallescens*; Sutton 1973) in having more variable conidial septation, conidia with a long apical cell, the production of *Selenosporella*-like synanamorph on conidia (*E. acutispora* and *E. filiformis*), evenly development in the dimorphic conidia in being obclavate and Y-shaped (*E. sporoprolifera*). However, these new taxa show the important characteristics of *Endophragmiella* by their presence of solitary, holoblastic, acrogenous, septate, dematiaceous conidia with a small basal frill from monoblastic, integrated, terminal, percurrently extending conidiogenous cells on mononematous, simple or branched, dematiaceous conidiophores and rhexolytic conidial secession that warrant their inclusion in *Endophragmiella.*

Endophragmiella species with a long acerose appendage include *E. filiformis, E. fusiformis, E. nanlingensis, E. pulchra, E. rostrata, E. sporoprolifera, E. unisetulata,* and *E. variabilis* (Matsushima 1975; Hughes 1979; Kirk 1982b, 1985; Castañeda-Ruíz 1988; Wu & Zhuang 2005, Ren & al. 2011) are somewhat similar to some taxa in *Alternaria* sect. *Porri* D.P. Lawr. & al. (see Woudenberg & al. 2014) by possessing obclavate, multiseptate and pigmented conidia with a long filiform beak at the apical end on simple or branched and cylindrical conidiophores. However, conidia of these *Endophragmiella* are phragmosporous, solitary, and euseptate on terminal and percurrently extending conidiogenous cells, while conidia of *Alternaria* sect. *Porri* are dictyosporous or phragmosporous, solitary or arranging in short acropetal chains, and disto- and euseptate on terminal or intercalary and non-extending conidiogenous cells. Conidial secession of *Endophragmiella* is rhexolytic, while that of *Alternaria* sect. *Porri* is schizolytic (Seifert & al. 2011, Lawrence & al. 2013).

Endophragmiella obpyriformis and *E. sporoprolifera* share an interesting feature of their conidia, which show a lateral percurrently extending conidiophore while still attached to the conidiogenous cell. This conidial feature closely resembles those of *E. corticola* P.M. Kirk, *E. fatrensis* Hol.-Jech., *E. hymenochaeticola* S. Hughes, and *E. lignicola* S. Hughes (Hughes 1978, 1979; Kirk 1982a; Holubová-Jechová 1986). Kirk (1982a) divided the characteristic of conidiophore proliferation into two types: first, the typical proliferation type, as seen in many *Endophragmiella* species in which the conidiophore proliferates

through the apex of the terminal conidiogenous cell; second, the lateral proliferation type, as seen in *E. corticola*, in which conidiophore proliferation arises from the lateral wall of the terminal conidiogenous cell. Unlike the two types mentioned by Kirk (1982a), the proliferation of *E. obpyriformis* and *E. sporoprolifera* occurs at the basal cell of the conidium that is still adhered to the conidiophore.

Endophragmiella appears morphologically similar with other hyphomycete genera in shape, pigmentation, conidial ontogeny and rhexolytic conidial secession to several existing genera associated with such as *Acrophragmis* Kiffer & Reisinger, *Blastophragmia* Jian Ma & al., *Chaetendophragmia* Matsush., *Stratiphoromyces* Goh & K.D. Hyde and *Teratosperma*. However, *Acrophragmis* distinguishes by its conidia presenting a ring of small knobs at central cell (Kiffer & Reisinger 1970); *Blastophragmia* distinguishes by its conidia bearing setulae (Ma & al. 2021b); *Chaetendophragmia* distinguishes by its conidia containing lateral appendages at the middle cells (Matsushima 1971); *Stratiphoromyces* distinguishes by its conidiogenous cells with crowded repeating percurrent extensions that lead to multilayers of wall remnants at the apex of the conidiophores producing conidia with setulae aggregating in a mass (Goh & Hyde 1998) and *Teratosperma* distinguishes by its conidia with lateral branches arising from the basal cell (Sydow & Sydow 1909). None of these similar genera have DNA sequence data for elucidating their phylogenetic relationships and therefore the differences in generic circumscription between these genera cannot be examined by molecular data at the moment.

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Diaporthe elaeagni-confertae and *D. fohaiensis* spp. nov. from Yunnan Province, China

SHENG-TING HUANG[1#], JI-WEN XIA[2#], XIU-GUO ZHANG[2], WEN-XIU SUN[1*]

[1] *College of Life Sciences,Yangtze University, Jingzhou, Hubei 434023, China*

[2] *Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests, College of Plant Protection, Shandong Agricultural University, Taian, Shandong 271018, China*

[#] *These authors contributed equally to this work*

[*] CORRESPONDENCE TO: *wenxiusun@163.com*

ABSTRACT—Three *Diaporthe* strains were isolated from the leaf spots of diseased *Elaeagnus conferta* and *Lithocarpus fohaiensis* in Yunnan Province, China. Based on a combination of morphology and a five loci phylogeny (ITS, tub2, tef1-α, cal, and his3) indicated that these strains represented two new species, *Diaporthe elaeagni-confertae* and *D. fohaiensis*.

KEY WORDS—asexual *Ascomycota*, *Diaporthales*, *Diaporthaceae*, phylogeny, taxonomy

Introduction

Diaporthe Nitschke (*Diaporthaceae, Diaporthales*) was described by Nitschke (1870). Rossman & al. (2015) pointed out that the name *Diaporthe* has priority over *Phomopsis* (the name previously used for asexual morphs) in the context of the one fungus – one name initiative, and most accepted species have a *Diaporthe* name. *Diaporthe* is mainly characterised by immersed ascomata and erumpent pseudostroma with elongated perithecial necks; asci are unitunicate, clavate to cylindrical; ascospores are fusoid, ellipsoid to cylindrical, hyaline, biseriate to uniseriate in the ascus, sometimes with appendages (Udayanga & al. 2011; Senanayake & al. 2017, 2018). The asexual morph (*Phomopsis*) is mainly characterised by ostiolate conidiomata with cylindrical phialides that produce up to three forms of hyaline, aseptate conidia called α-conidia,

β-conidia, and γ-conidia (Udayanga & al. 2011; Gomes & al. 2013). The γ-conidia are rarely produced and difficult to observe (Gomes & al. 2013; Guarnaccia & Crous 2017; Guo & al. 2020). Most *Diaporthe* species have been reported as destructive plant pathogens, innocuous endophytes isolated from asymptomatic plant tissues, saprophytes that colonize and degrade dead leaves, bark, and twigs, or pathogenic fungi that cause health problems in humans and other mammals (Murali & al. 2006; Udayanga & al. 2012; Gomes & al. 2013; Ménard & al. 2014; Guarnaccia & al. 2016, 2018; Torres & al. 2016; Senanayake & al. 2018). Currently, of more than 1100 epithets listed in *Diaporthe* by Index Fungorum (23 October 2022), only one-fifth has been well-studied with ex-type cultures and supplementary DNA barcodes (Guo & al. 2020; Yang & al. 2020). For phylogenetic analysis of *Diaporthe* species, a five loci dataset (internal transcribed spacer [ITS] / beta-tubulin [tub2] / translation elongation factor 1-α [tef1-α] / calmodulin [cal] / histone H3 [his3]) is the optimal combination of species delimitation revealed by Santos & al. (2017).

Yunnan Province, located in southeastern China, has a unique geography with distinct climatic coniditions and abundant plant resources. Its ecological environment is conducive to the growth of unusual microbial species, which creates a higher species diversity than elsewhere in China. During our study of *Diaporthe* species in Yunnan Province, two new *Diaporthe* species were identified based on morphological characters and phylogenetic analysis.

Materials & methods

Isolates and Morphological analysis

Diseased leaves were collected from *Elaeagnus conferta* and *Lithocarpus fohaiensis* in Menghai county, Yunnan Province, China. Tissue pieces (5 × 5 mm) were taken from the margin of leaf lesions and surface-sterilized by consecutively immersing in 75% ethanol solution for 1 min, 5% sodium hypochlorite solution for 30 s, and then rinsing three times with sterile distilled water for 1 min. The pieces were dried with sterilized paper towels and then placed on potato dextrose agar (PDA) (Cai & al. 2009). All the PDA plates were incubated at 25°C for 2–4 d, and from which the colonies periphery were picked out and inoculated onto new PDA plates. Colonies photos in 7 d and 15 d were taken by digital camera (Powershot G7X mark II). Micromorphological characters were observed using Olympus SZX10 stereomicroscope and Olympus BX53 microscope, all fitted with Olympus DP80 high definition colour digital cameras to photo-document fungal structures. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. The studied specimens are deposited in the Herbarium of Plant Pathology, Shandong Agricultural University (HSAUP). Ex-type cultures are deposited in the Shandong Agricultural University Culture Collection

(SAUCC), Taian, Shandong Province, China. Taxonomic information of new species were submitted to MycoBank (http://www.mycobank.org).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from colonies grown on PDA, using the CTAB method (Guo & al. 2000). The five gene regions were amplified and sequenced with the following primer pairs: ITS with ITS4/ITS5 (White & al. 1990); tub2 with Bt2a/Bt2b (Glass & Donaldson 1995); tef1-α with EF1-728F/EF1-986R (Carbone & Kohn 1999); cal with CAL-228F/CAL-737R (Carbone & Kohn 1999); and his3 with CYLH3F/H3-1b (Glass & Donaldson 1995; Crous & al. 2004). PCR was performed using an Eppendorf Master Thermocycler (Hamburg, Germany). Amplification reactions were performed in a 25 μL reaction volume which contained 12.5 μL Green Taq Mix (Vazyme, Nanjing, China), 1 μL of each forward and reverse primer (10 μM) (Biosune, Shanghai, China), and 1 μL template genomic DNA in amplifier, and were adjusted with distilled deionized water to a total volume of 25 μL. PCR parameters were as follows: 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at a suitable temperature for 30 s, extension at 72°C for 1 min and a final elongation step at 72°C for 10 min. Annealing temperature for each gene was 55°C for ITS, 60°C for tub2, 52°C for tef1-α, 54°C for cal, and 57°C for his3. PCR products were estimated visually by staining with GelRed after 1% agarose gel electrophoresis. Sequencing was done bi-directionally by the Biosune Company Limited (Shanghai, China). Consensus sequences were obtained using MEGA 7.0 (Kumar & al. 2016). Sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

Novel sequences generated from our three strains in this study, and all available reference sequences of *Diaporthe* species were downloaded from NCBI's GenBank and alignments of the individual locus determined using MAFFT v. 7.110 (Katoh & al. 2019), and manually corrected where necessary. To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each locus (data not shown) and then as combined analyses of the five loci. Phylogenetic analyses were based on Maximum likelihood (ML) and Bayesian Inference (BI) for the multi-locus analyses. ML and BI were run on the CIPRES Science Gateway portal (https://www.phylo.org/; Miller & al. 2012) using RaxML v. 8.2.10 (Stamatakis 2014) and MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003), respectively. For ML analyses the default parameters were used and BI was carried out using the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included five parallel runs of 5,000,000 generations, with the stop rule option and a sampling frequency of 1000 generations. The burn-in fraction was set to 0.25 and posterior probabilities (PP) were determined from the remaining trees. The resulting trees were plotted using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CS v. 5.

TABLE 1. Species and sequences used in phylogenetic analyses. New sequences in bold. Isolates marked with "*" are ex-type or ex-epitype strains.

SPECIES NAME	VOUCHER	HOST/SUBSTRARE	GENBANK ACCESSION NUMBER					
			ITS	tub2	tef1-α	cal	his3	
Diaporthe biguttulata	CGMCC 3.17248* = ZJUD 47	Citrus limon	KJ490582	KJ490403	KJ490461	—	KJ490524	
	CGMCC 3.17249 = ZJUD 48	Citrus limon	KJ490583	KJ490404	KJ490462	—	KJ490525	
D. brasiliensis	CBS 133183*	Aspidosperma tomentosum	KC343042	KC344010	KC343768	KC343284	KC343526	
D. caatingaensis	URM 7486* = CBS141542	Tacinga inamoena	KY085926	KY115600	KY115603	KY115597	KY115605	
D. cinnamomi	CFCC 52569	Cinnamomum sp.	MH121504	MH121586	MH121546	—	MH121464	
	CFCC 52570	Cinnamomum sp.	MH121505	MH121587	MH121547	—	MH121465	
D. citriasiana	ZJUD81	Citrus paradisi	KJ490616	KJ490437	KJ490495	—	KJ490558	
	ZJUD82	Citrus paradisi	KJ490617	KJ490438	KJ490496	—	KJ490559	
D. discoidispora	ZJUD87 = CGMCC 3.17254	Citrus sinensis	KJ490622	KJ490443	KJ490501	—	KJ490564	
D. elaeagni-confertae	**SAUCC194.47***	**Elaeagnus conferta**	**MT822575**	**MT855772**	**MT855888**	**MT855656**	**MT855544**	
D. fohaiensis	**SAUCC194.113***	**Lithocarpus fohaiensis**	**MT822641**	**MT855838**	**MT855953**	**MT855720**	**MT855608**	
	SAUCC194.115	**Lithocarpus fohaiensis**	**MT822643**	**MT855840**	**MT855955**	**MT855722**	**MT855610**	
D. malorum	CAA752* = CBS 142383	Malus domestica	KY435643	KY435671	KY435630	KY435661	KY435651	
	CAA740	Malus domestica	KY435642	KY435670	KY435629	KY435660	KY435650	

Species name	Voucher	Host/Substrate	Genbank accession number				
			ITS	tub2	tef1-α	cal	his3
D. minusculata	CGMCC 3.20098*	on decaying branch	MT385957	MT424712	MT424692	—	—
	GZCC 19-0345	on decaying branch	MT797184	MT793038	MT793027	—	—
D. oxe	CBS 133186*	*Maytenus ilicifolia*	KC343164	KC344132	KC343890	KC343406	KC343648
	CBS 133187	*Maytenus ilicifolia*	KC343165	KC344133	KC343891	KC343407	KC343649
D. paranensis	CBS 133184*	*Maytenus ilicifolia*	KC343171	Kc344139	KC343897	KC343413	KC343655
D. siamensis	MFLUCC 10-0573a	*Dasymaschalon* sp.	JQ619879	JX275429	JX275393	JX197422	—
	MFLUCC 10-0573b	*Dasymaschalon* sp.	JQ619880	JX275430	JX275395	JX197423	—
D. tarchonanthi	CPC 37479 = CBS 146073	*Tarchonanthus littoralis*	MT223794	MT223733	—	—	MT223759
D. yunnanensis	CGMCC 3.18289* = LC6168	*Coffea* sp.	KX986796	KX999228	KX999188	KX999290	KX999267
	LC8106	*Coffea* sp.	KY491541	KY491561	KY491551	KY491571	—
	LC8107	*Coffea* sp.	KY491542	KY491562	KY491552	KY491572	—
Diaporthella corylina	CBS 121124	*Corylus* sp.	KC343004	KC343972	KC343730	KC343246	KC343488

Phylogentic results

Three *Diaporthe* strains isolated from plant hosts were sequenced. *Diaporthe* spp. was analysed by using multi-locus data from 26 isolates including *Diaporthella corylina* (CBS 121124) as outgroup. A total of 2658 characters including gaps were obtained in the phylogenetic analysis: ITS, 1–609; tub2, 610–1185; tef1-α, 1186–1601; cal, 1602–2141; and his3, 2142–2658. Of these characters, 1683 were constant, 365 were variable and parsimony-uninformative, and 610 were parsimony-informative. For the BI and ML analyses, GTR+I+G for ITS and his3, HKY+G for tub2 and cal, GTR+G for tef1-α were selected and incorporated into the analyses. The topology of ML tree confirmed the tree topologies obtained from the BI analyses, and therefore, only the ML tree is presented (Fig. 1).

ML bootstrap support (BS) ≥50% and Bayesian posterior probability (PP) ≥0.90 are shown as first and second position. The 26 strains were assigned to 16 species clades, based on the five gene loci phylogeny (Fig. 1). In this tree, our three new strains were assigned to two novel species, *Diaporthe elaeagni-confertae* and *D. fohaiensis*.

Taxonomy

Diaporthe elaeagni-confertae S.T. Huang, J.W. Xia, X.G. Zhang & W.X. Sun,
sp. nov.   Fig. 2

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Differs from *Diaporthe siamensis* by its larger β-conidia; and from *D. fohaiensis* by its production of α-conidia and its shorter β-conidia.

Type: China, Yunnan Province, Menghai county, Nannuoshan, on infected leaves of *Elaeagnus conferta* Roxb. (*Elaeagnaceae*), 19 April 2019, S.T. Huang (**Holotype**, HSAUP194.47; ex-type living culture, SAUCC194.47; GenBank MT822575, MT855772, MT855888, MT855656, MT855544).

Etymology: refers to the host *Elaeagnus conferta*, on which the fungus was collected.

Colonies covering dish after 15 days in the dark at 25°C on PDA. Cottony with abundant aerial mycelium, and concentric zonation, both white on surface side and reverse. Conidiomata pycnidial, subglobose to globose, multi-pycnidia aggregated in groups, gray to black, erumpent, coated with white and grayish hyphae, thick-walled, exuding creamy conidial droplets from ostiole. Paraphyses not observed. Conidiophores hyaline, smooth, rarely septate, branched, densely aggregated, cylindrical, straight to slightly sinuous, 8.0–13.5 × 2.0–2.5 µm. Conidiogenous cells 12.5–15.5 × 1.5–2.2 µm, cylindrical, guttulate, terminal, tapering towards the apex. Alpha conidia, biguttulate or
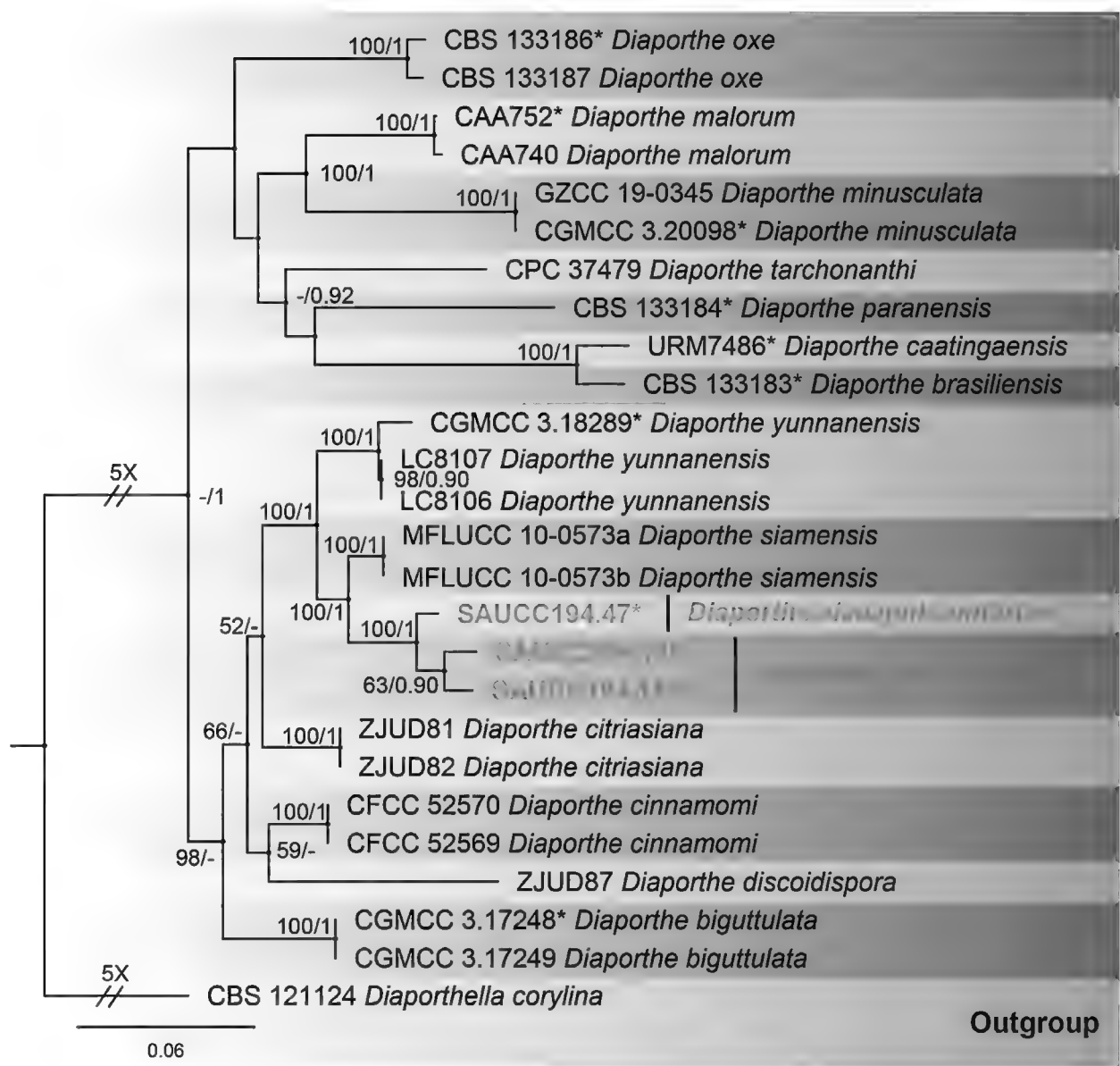
FIG. 1. Phylogram of *Diaporthe* based on combined ITS, tub2, tef1-α, cal, and his3 genes. Bootstrap support values are shown for ML ≥50% and BI ≥0.90. Strains marked with "*" are ex-type or ex-epitype. Our three new strains are in red. Some branches were shortened to fit them to the page – these are indicated by two diagonal lines accompanied by the length reduction factor.

eguttulate, hyaline, smooth, aseptate, ellipsoidal, oval, 4.5–6.5 × 1.8–2.8 μm (mean = 5.9 × 2.3 μm, n = 20). BETA CONIDIA abundant in culture, hyaline, aseptate, filiform, mostly curved through 0–90° at the apex, tapering towards the apex, base truncate, 29.5–36.0 × 1.0–1.2 μm (mean = 30.5 × 1.2 μm, n = 20). GAMMA CONIDIA not observed. Sexual morph: undetermined.

COMMENTS—Phylogenetic analysis of a combined five loci showed that *Diaporthe elaeagni-confertae* (strain SAUCC194.47) formed an independent clade (FIG. 1) and is phylogenetically distinct from *D. fohaiensis* and *D. siamensis* (Gao & al. 2016). This species can be easily distinguished from *D. siamensis* by 64 nucleotides difference in the concatenated alignment (16/491 in ITS;
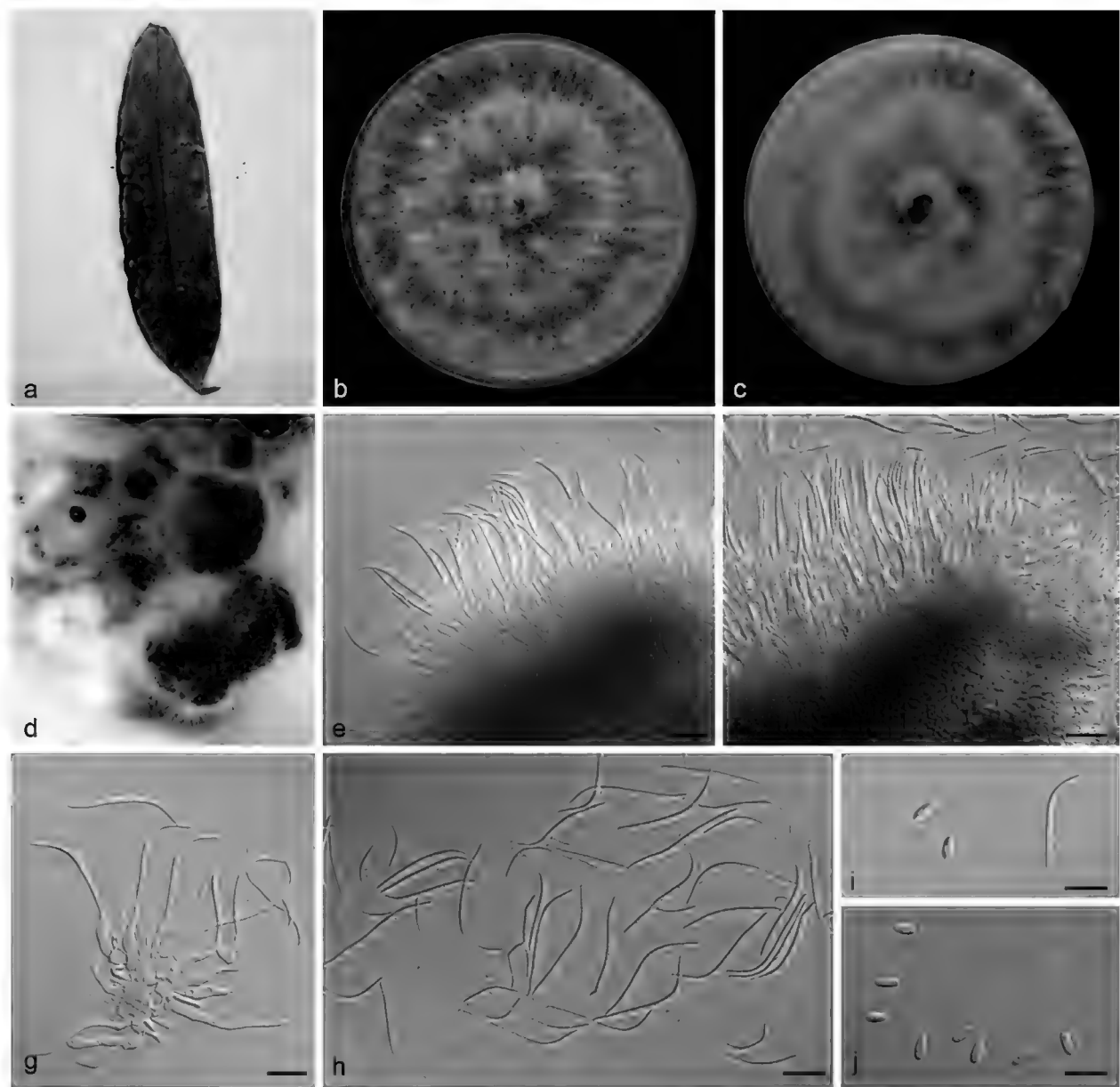
FIG. 2. *Diaporthe elaeagni-confertae* (ex-holotype, SAUCC194.47). a. Leaves of host plant; b. Surface of colony after 15 days on PDA; c. Reverse of colony after 15 days on PDA; d. Conidiomata sporulating on PDA margin; e–g. Conidiogenous cells with developing conidia; h. β-conidia; i. α-conidia and β-conidia; j. α-conidia. Scale bars: e–j = 10 μm.

8/446 in tub2; 24/346 in tef1-α; and 16/442 in cal); and from *D. fohaiensis* by 46 nucleotides difference concatenated alignment (10/556 in ITS; 9/499 in tub2; 4/340 in tef1-α; 16/467 in cal; and 7/444 in his3). Morphologically, *D. siamensis* differs by its smaller β-conidia (15.0–18.0 × 1.5–2.0 μm), and *D. fohaiensis* differs by its longer β-conidia (33–37.5 μm long) and its lack of α-conidia.

Diaporthe fohaiensis S.T. Huang, J.W. Xia, X.G. Zhang & W.X. Sun, **sp. nov.**

MB 839335   FIG. 3

Differs from *Diaporthe elaeagni-confertae* by its lack of α-conidia, its longer β-conidia, and its shorter conidiogenous cells.
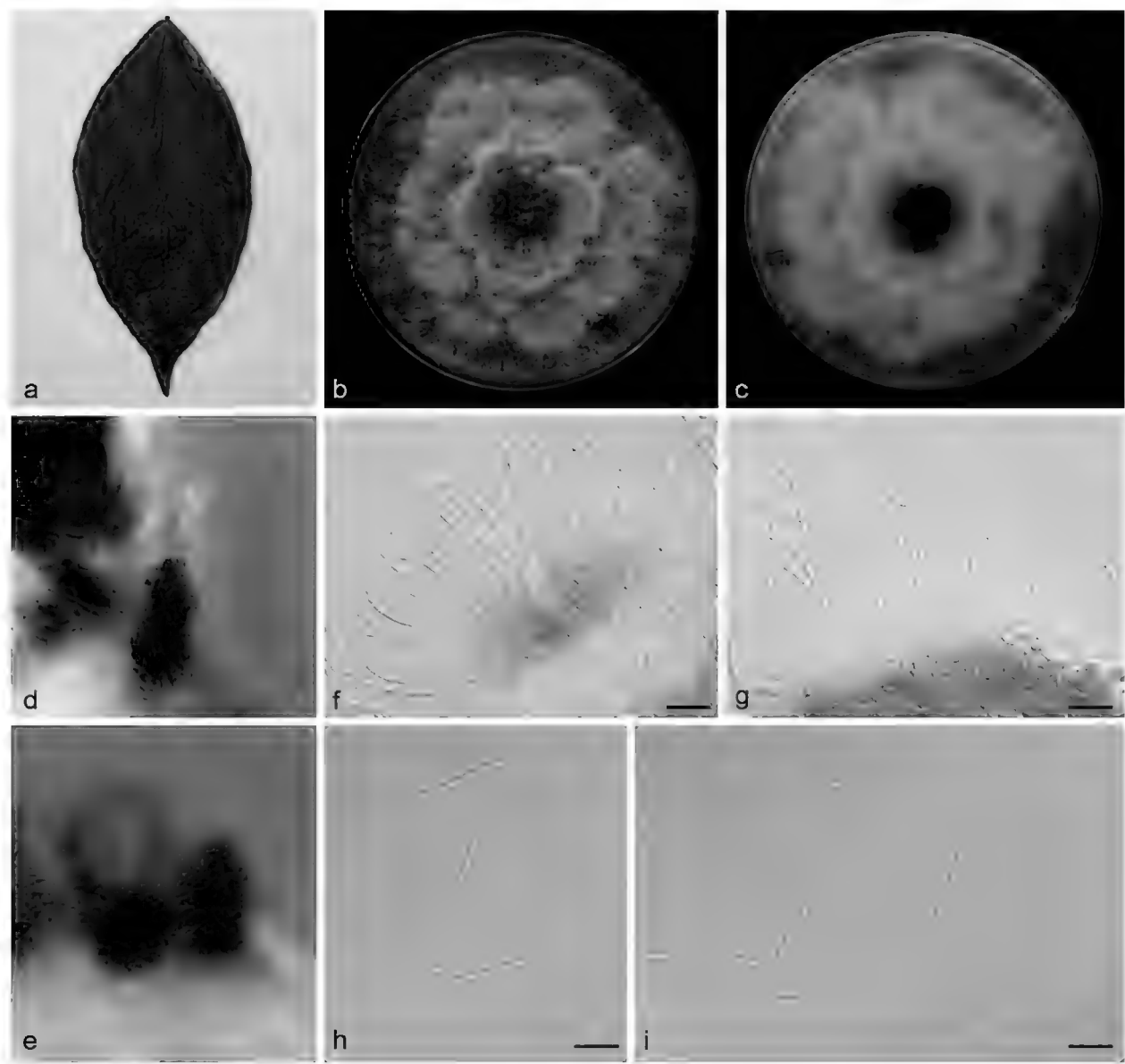
Fɪɢ. 3. *Diaporthe fohaiensis* (ex-holotype, SAUCC194.113). a. Leaves of host plant; b. Surface of colony after 15 days on PDA; c. Reverse of colony after 15 days on PDA; d, e. Conidiomata sporulating on PDA margin; f, g. Conidiogenous cells with developing conidia; h, i. β-conidia. Scale bars: f–i = 10 μm.

Type: China, Yunnan Province, Menghai county, Nannuoshan, on infected leaves of *Lithocarpus fohaiensis* (Hu) A. Camus (*Fagaceae*), 19 April 2019, S.T. Huang (**Holotype**, HSAUP194.113; ex-type living culture, SAUCC194.113; Genbank MT822641, MT855838, MT855953, MT855720, MT855608).

Etymology: refers to the epithet of the host *Lithocarpus fohaiensis* on which the fungus was collected.

Cᴏʟᴏɴɪᴇꜱ covering dish after 15 days in the dark at 25°C on PDA. Cottony with abundant aerial mycelium, hyphae white on surface side, lobate in the margin, with pare brown concentric ring of dense hyphae on reverse side. Cᴏɴɪᴅɪᴏᴍᴀᴛᴀ pycnidial, subglobose to globose, scattered or multi-pycnidia

aggregated in groups, pale brown to black, erumpent, coated with white and gray hyphae, thick-walled, exuding creamy conidial droplets and spiral conidial cirrus from ostiole. Conidiophores hyaline, smooth, rarely septate, branched, densely aggregated, cylindrical, straight to slightly sinuous, 4.5–9.5 × 1.8–3.0 µm. Conidiogenous cells, cylindrical, terminal, rarely guttulate, tapering towards the apex, 7.8–11.5 × 1.5–2.5 µm. Alpha conidia and gamma conidia not observed. Beta conidia abundant in culture, hyaline, aseptate, filiform, mostly curved through 90–180° at the apex, tapering towards both ends, base truncate, 33–37.5 × 1.0–1.5 µm (mean = 35.5 × 1.3 µm, n = 20). Sexual morph: undetermined.

Additional specimen examined: **CHINA, Yunnan Province, Menghai county**, Nannuoshan, on diseased leaves of *Lithocarpus fohaiensis*, 19 April 2019, S.T. Huang (HSAUP194.115; living culture SAUCC194.115; Genbank MT822643, MT855840, MT855955, MT855722, MT855610).

Comments—Phylogenetic analysis of a combined five loci showed that *Diaporthe fohaiensis* (strains SAUCC194.113 and SAUCC194.115) formed an independent clade (Fig. 1), and is phylogenetically distinct from *D. elaeagni-confertae*. This species can be easily distinguished from *D. elaeagni-confertae* by 46 nucleotides difference in the concatenated alignment (10/556 in ITS; 9/499 in tub2; 4/340 in tef1-α; 16/467 in cal; and 7/444 in his3). Morphologically, *D. fohaiensis* differs from *D. elaeagni-confertae* which has α-conidia, shorter β-conidia (29.5–36.0 µm long), and longer conidiogenous cells (12.5–15.5 µm long).

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MYCOTAXON

Regional annotated mycobiota new to the Mycotaxon website

ABSTRACT—Mycotaxon is pleased to add three new annotated species distribution lists to our collection of previously posted free-access fungae. These checklists may be downloaded from our website via http://www.mycotaxon.com/mycobiota/ .

NORTH AMERICA

United States

THERESA KADISH, JULES AMANITA, KATHLEEN WHITE, TYLER G. VINCA, DREW ACOFF, REBECCA GRABARCHUK, MICHAEL ABDALLAH, & KENNEDY WHITE. Macrofungi from the East Brook Valley of Delaware County New York, USA. 42 p.

ABSTRACT—This study surveys the area of East Brook Farm, Walton, NY, for its macrofungi biodiversity in seven site locations over a five week period from July – August 2021. Macrofungi specimens were collected, photographed, described, and dehydrated for preservation in herbarium archives stored at Binghamton University. A total of 41 species were collected and described. Species descriptions, location details, and phylogenetic information are provided in this survey. The importance of citizen science and collaborative surveying is also discussed.

KEYWORDS —biodiversity, collaborative, citizen science, survey

D.S. NEWMAN, R.A. CRONCE, E.D. DOMBKOWSKI, C.B. DOOLEY. Checklist of fungi from the first Richard P. Korf Memorial North American Ascomycete Foray and Purchase Knob AscoBlitz.

ABSTRACT—Held in honor of legendary Cornell discomycetologist and Mycotaxon co-founder, the Richard P. Korf Memorial North American Ascomycete Foray (colloquially referred to as "The Korf Foray") and Purchase Knob AscoBlitz are believed to represent the first ascomycete-focused forays ever conducted in the United States. Modeled after the meetings of the Pacific Northwest Key Council and the ascomycete field workshops

of northern Europe, both events took place in mid-August of 2023 in western North Carolina, at the Highlands Biological Station and Appalachian Highlands Science Learning Center, respectively. Each were made possible by generous contributions from the Korf family and Discover Life in America.

The Korf Foray attracted a total of 22 participants (including four scholarship recipients) from the United States and Germany, with seven continuing on to the AscoBlitz, resulting in the generation of approximately 275 fungal records supported by 211 vouchered collections. Among these are represented at least 88 genera, 62 families, 29 orders and 11 classes within the Ascomycota. The majority of collections will be deposited at the Cornell Plant Pathology Herbarium (CUP), with specimen data available in the online checklist which will be updated periodically.

Corresponding macro- and micro-photography are available on iNaturalist, linked from each collection record in the checklist (https://www.inaturalist.org/projects/the-richard-p-korf-memorial-north-american-ascomycete-foray-2023).

KEYWORDS — diversity, bioblitz, North Carolina, Highlands, parataxonomy, events, ascomycetology, education

SOUTH AMERICA

Brazil

LOPES, CASSIANE FURLAN, ALICE LEMOS COSTA, MARINES ÁVILA HEBERLE, KAMILLE RODRIGUES FERRAZ, JAIR PUTZKE. Muscicolous Agaricales (Basidiomycota: Agaricomycetes) found in Brazil.

ABSTRACT— Agaricomycetes muscicolous fungi have been little studied in Brazil, both in their taxonomy and in ecology. Thus, here we present a compendium of species of muscicolous Agaricales found in Brazil based on a bibliographic review. To assist the taxonomic identification of the group, a dichotomous identification key is also proposed. Based on the literature review, 19 species of muscicolous Agaricales were cataloged as occurring in Brazil. Among the species dealt with here, nine are identified as moss parasites. This demonstrates a great gap in the scientific knowledge of this subject in Brazil, which needs a broad deepening to better understand the diversity of these interactions and their ecology.

KEYWORDS — Bryophilous fungi, Bryophyta, parasitic associations.
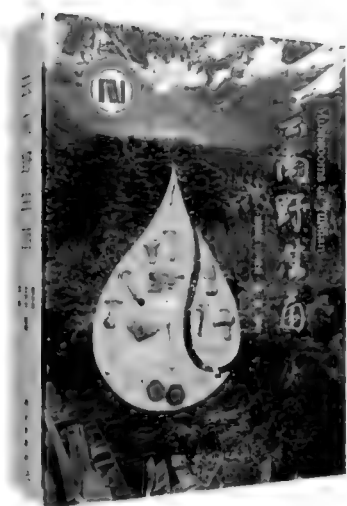
Book announcement

ELSE C. VELLINGA[1*]

[1] *UC Herbarium, Berkeley, CA 94720, U.S.A.*

[*] CORRESPONDENCE TO: *ecvellinga@comcast.net*

General

Mushrooms of Yunnan. By Z.-L. Yang, X.-H. Wang & G. Wu. 2022. Science Press of Beijing. www.sciencep.com. ISBN 978-7-03-72402-1. 368.00 元. 378 pp. in Chinese.

This book gives a first introduction to the rich mycoflora of Yunnan, in southwestern China. Yunnan stretches from the (sub)tropics in the south (bordering Myanmar and Laos) to the high mountain ranges in the northwest, near the border with Tibet. The huge plant and vegetation diversity, related to the variety in terrain, has also caused a unique and diverse funga, which is still insufficiently known. 548 species were selected to represent the diversity of Basidiomycota and mushroom forming Ascomycota in this book. Each species is illustrated with an in-situ photo, and short descriptions (in Chinese) accompany the photos. To give you an idea about the content, more than 80 species of boletes are included, and 37 milkcap species in the genera *Lactarius* and *Lactifluus*.

The book starts with very short introductions to the mushrooms of Yunnan and mushroom morphology, followed by the bulk, i.e., the photos and descriptions of the selected species in alphabetical order by genus, whereas references, a list

of the photographers and indices to the Chinese and scientific names make up the rear end of the book. As a non-Chinese speaker I cannot comment on the quality of the descriptions.

It is a pleasure to leaf through the book, as the quality of the photos is high, and the photos transplant me to this area again. I had the pleasure of two short mycological visits to Yunnan, five and 15 years ago.

The only draw back of the book is its girth and weight; it is too big and heavy to pack into a backpack or carry-on.

SCOTT A. REDHEAD[1], JOSEPH F. AMMIRATI[2], NONI KORF, SHAUN R. PENNYCOOK[3]

[1]*Canadian National Mycological Herbarium, Ottawa Research and Development Centre, Science and Technology Branch, Agriculture and Agri-Food Canada,*
Ottawa, ON, Canada, K1A 0C6
[2]*Department of Biology,*
University of Washington, Seattle, Washington 98195, USA
[3]*Manaaki Whenua–Landcare Research, Private Bag 92170,*
Auckland 1072, New Zealand

Lorelei Louise Norvell (1943 – 2023)

Dr. Lorelei L. Norvell, the Editor-in-Chief of **Mycotaxon** (Vols. 89–137 [2004–22]) for the past 20 years, passed away 4 August 2023, at her home in Portland, Oregon, following a prolonged battle with cancer. Lorelei dedicated her last few months to finalizing two delayed issues of the 2022 volume of the journal thus closing a significant chapter in the journal's history. Fittingly, the final issue, which was partly edited by Lorelei, is dedicated to her memory.

Lorelei took over the editorship of **Mycotaxon** from Dr. Pavel Lizoň in 2003 and helped guide the journal through a transformative modernization to digital, online publication. Lorelei used her language and editorial skills to help non-English speaking authors by improving their English and resolving complex sentence structures for authors not writing in their first language. This was highly appreciated by hundreds of contributors. She did this by drawing upon her understanding of both mycology and the nuances of linguistics. Before becoming a mycologist, Lorelei had studied several modern languages at Knox College, graduating with an A.B. in Germanic languages from Knox College (Phi Beta Kappa and magna cum laude) in 1964, and Russian, Serbo-Croatian,

and other Slavic languages (obtaining an MA from the University of Texas at Austin in 1969). She was at ease in languages such as German, the ancestral tongue of her father, French, and Russian among others. She developed a significant Russian mycological literature library. Lorelei had great patience and was generous, one could say overly generous, with time spent assisting new scientists and new authors. Additionally, she understood the complexity of the **International Code of Nomenclature for algae, fungi, and plants**, and until her death served on the Nomenclature Committee for Fungi, including being its Secretary from 2008–13. Lorelei developed a close working relationship with Dr. Shaun Pennycook, who was appointed as Nomenclature Editor of **Mycotaxon** in 2005 to lighten her workload. Lorelei was foremost a team player and enthusiastic promoter of science at the local, regional, and global levels.

There is no doubt that Lorelei Norvell was an exceptional and extremely bright child and stood out academically at an early age. As an only child she grew up being doted upon by her parents, David and Betty Lehwalder. Born in Oakland, California, the family moved repeatedly, and her schooling shifted in synch with the career of her father, a chemist in the oil industry, from Oakland, CA, to Houston, TX, where she first appeared in the news (**Houston Chronicle**) as an eager 10 year old student in 1953 boarding a school bus, then Edwardsville, IL, and finally, Roxana, IL where she completed high school as class valedictorian, summa cum laude. Next Lorelei obtained her first graduate degree, an A.B. from Knox College, Galesburg, IL in 1964. Each summer was spent in Montana primarily with her mother and relatives where she developed a keen interest in nature.

Lorelei married her late husband G. Todd Norvell in 1964. Todd served in the compulsory Reserve Officer Training Corps (ROTC), became a Distinguished Military Graduate, and subsequently entered the U.S. Army later that year as the Vietnam War effort draft ramped up. Following his career path, Lorelei and Todd first moved to Columbus, GA near Fort Benning (now Fort Moore), then to Killeen, TX near Fort Hood, and during his meritorious overseas deployment and training at Fort Holabird, MD, Lorelei moved to Godfrey, IL near her childhood town of Roxana, to enter the Southern Illinois University Edwardsville, where she obtained her high school teacher accreditation. She had an insatiable appetite for education. Following Todd's return they moved to Austin, TX so that he could enroll in the University of Texas Law School. There she continued her education at the same university, this time in languages and with summers spent at the Russian Summer Institute in Lawrence, KS,

graduating in 1969. The couple next moved to Denver, CO where Todd clerked at a Denver law firm, and soon after they moved to Portland, OR, where Todd joined a prestigious law firm.

Having settled in Oregon, Todd rose in the ranks to become a senior partner, and they designed and built a spectacularly unique house later featured in the local **Sunset Magazine.** Lorelei started a leaded glass studio in their home in 1970 that was featured in **The Oregonian** newspaper that in 1975 billed her as a 'dilettante' stained-glass artist.

Following the birth of their son, Forrest, in 1972, Lorelei became interested in mushrooms and joined the Oregon Mycological Society (OMS) marking a turning point in her life. By 1975 she had become the President of OMS and had begun a comprehensive file on all Oregon and Pacific Northwest (PNW) mushrooms. Following the birth of their second son, Owen in 1978, Lorelei was elected chair of the Skyline School Local School Advisory Committee. In 1980 they experience the eruption of Mt. St. Helen's in nearby Washington state, which covered Portland and their home in volcanic ash.

one of Lorelei's botanical stained glass windows
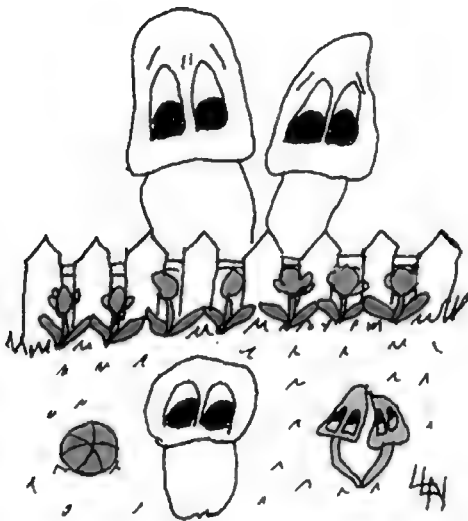
The Ipecac Crew

from "Poisonings I have Known" reprinted with permission from *Mushroom, The Journal of Wild Mushrooming*: Autumn 1986 4(4)

Beginning in 1983 Lorelei began publishing a 14 year long run of humorous, educational articles, each illustrated by her uniquely whimsical, big-eyed mushroom cartoon characters. She often would sign L^3N which stood for L- cubed or LLLN, as she was proud of her birth name 'Lehwalder' combined with her given names Lorelei Louise.

By 1986 she was again President of OMS, Chair of the OMS toxicology committee, and one of the most knowledgeable amateur mycologists in the PNW. Also in 1986, the OMS with Lorelei Norvell being a major participant, initiated the Oregon *Cantharellus* Study Project, which was to last for 10 years. Contemporaneously with her interest in fungi and stained glass she became serious about the game Scrabble, eventually entering competitive Scrabble and ultimately competing in the annual Scrabble

Open in Las Vegas around 1987. She and Todd, both being gifted individuals, also took up the guitar, continually competing with one another to be the best.

Joe Ammirati (professor) and Lorelei Norvell (student) in Joe's office,
University of Washington about 1994 (photo by S.A. Redhead)

Having met several prominent professional mycologists through her involvement with the OMS and the PNW mushroom Key Council, Lorelei, then the Vice President, later (1988) President, approached J. Ammirati in 1987, to explore the possibility of studying for a doctorate in mycology at the University of Washington. She was told that she needed a Bachelor's degree in science to pursue the program so she promptly enrolled in Portland State University and earned a B.Sc. in botany (1990). Nothing was going to stand in her way! Lorelei then embarked on an 8-year long quest driving and living off and on campus between her home in Portland, OR and the University of Washington, Seattle, ultimately graduating in 1998 with a Ph.D. in mycology.

Lorelei at first focussed on the genus *Inocybe*, which had been studied by Dr. Dan Stuntz in Seattle, and she attended the 1990 North American Mycological Association (NAMA) foray in Whistler, BC, Canada, with Joe Ammirati where they met Scott Redhead who recently had published on *Phaeocollybia* in eastern Canada. Norvell and Redhead crossed paths again in 1991 at the Mycological Society of America (MSA) meeting in San Antonio, TX where she presented the OMS poster on the chanterelle project. Enter

Foray in British Columbia in 1991. Left to right: Shamin Gamiet, Andy MacKinnon, Shannon Berch, Lorelei Norvell, John Dennis, Brenda Callan, Paul Kroeger (photo by S.A. Redhead)

Lorelei and Scott Redhead in front of Scott's poster at the 5th International Mycological Congress, Vancouver, B.C., August, 1994

Sharmin Gamiet, who became a fellow student with Lorelei at UW in Seattle, and Shannon Berch from BC; knowing of Scott's interest in *Phaeocollybia*, Sharmin and Lorelei suggested that he attend the Foray of Western Mycologists (FOAM) that year on the Olympic Peninsula, since *Phaeocollybia* was far more common in old growth rains forests of the PNW than elsewhere in North

America. Several species of *Phaeocollybia* were found during FOAM, which prompted an impromptu decision by Sharmin to invite Lorelei to accompany Scott to her old growth Douglas Fir plots with a group of mycologists including Andy MacKinnon from Vancouver Island. In turn, Andy arranged that they immediately visit the lower Carmanah Valley on Vancouver Island, BC, via logging roads as it harboured some of Canada's oldest endangered conifer forests. *Phaeocollybia* had never been collected in BC previously, and they knew

Lorelei Norvell holding the first field find of an unnamed species, now *Phaeocollybia redheadii,* October 1991 in the old growth forests of the Carmanah Valley, Vancouver Island, BC

that the genus was fruiting that week just south of the valley across the Strait of Juan de Fuca. The field trip was a success and resulted in the discovery of two species, new to both BC and Canada. She published a humorous account "A Canadian Odyssey" in Mushroom (Spring 1992 10(2): 9–12) [see https://www.researchgate.net/publication/317000857_The_Canadian_Odyssey-25_years_later_Search_for_the_elusive_*Phaeocollybia*].

From that point on Lorelei suggested to her professor Joe Ammirati that she study *Phaeocollybia* instead of *Inocybe.* Joe Ammirati recalling his professor, Alexander H. Smith's interest in *Phaeocollybia,* knowing that the PNW was a hot spot for the genus, and that its limited taxonomic scope suited a Ph.D. project, he approved and guided the study. Worried that there might be insufficient material to complete a study of *Phaeocollybia,* a two month long survey with George Barron and Scott Redhead along coastal BC, WA, OR and northern California was undertaken in 1992 which yielded many well documented, photographed collections and species, several new to science and proved that Lorelei had an eye for the genus. Joe ensured that Lorelei used one of the latest DNA tools of the day in taxonomy, that of Restriction Fragment Length Polymorphism (RFLP) analyses. Joe also arranged that her external examiner was renowned molecular guru, Dr. David Hibbett. This launched Lorelei's studies leading to her becoming a global expert on *Phaeocollybia* and the foremost authority of the genus in North America.

As a result of her posters and publications on chanterelles and *Phaeocollybia,* Lorelei was awarded an MSA Graduate Fellowship Award in 1993. Also coincidental with Lorelei's residency at the University of Washington, Joe Ammirati and other biologists in the PNW were canvassed in 1993 by the US government's Forest Ecosystem Management Assessment Team (FEMAT) under President Bill Clinton's plan to free up or mitigate timber harvesting in the PNW. Joe was asked for a list of species of fungi, especially suspected rare or endangered taxa known either to be restricted to or frequenting more commonly in old growth PNW forests. Fortuitously, among the taxa placed on the initial lists were the supposedly rare *Phaeocollybia* and the then mysterious *Cantharellus formosus,* both then actively studied by Lorelei, the first as part of her doctoral study and the latter involving the OMS chanterelle project. The significance of these taxa and the necessity to first survey fungal herbaria for data and then survey forests for them, helped launch Lorelei Norvell's family contract business, Pacific Northwest Mycology Service, LLC, guided by her husband's business acumen and her adult son, Forrest's, information technology

skills. Via this company, based in her home, rather than a university research laboratory, Lorelei would assist the USA and Oregon governments, and publish major mycological treatises.

Aware of her writing and linguistic skills, Lorelei was appointed Editor of Inoculum, the MSA newsletter, by MSA President Jim Ginns, for 4 years beginning in 1998. She also began contract work for the US and regional governments surveying PNW mushrooms for land surveys. Simultaneously, she coordinated the Great Smoky Mountain National Park All-Taxa Biological Inventory Fungal Taxonomic Working Group (TWIG) between 1999–2000 in North Carolina and Tennessee, working with Drs. Ron Petersen and Karen Hughes. She was elected Secretary of MSA in 2000, serving for 4 years.

Lorelei in her home office in October 1998 (photo by S.A. Redhead)

Surviving her first bout of ovarian cancer treatment in 2001, she then became Editor of **Mycotaxon** in 2003. Lorelei believed in supporting taxonomy and appreciated both classical field-based and modern cutting-edge systematics and wished to keep the journal relevant and available to the world. Her appointment initiated a decades long series of consulting monthly phone calls with **Mycotaxon** founding editor, Richard Korf up until his death, and with the late editor Grégoire Hennebert. Concurrent with her editorship she continued to publish on nomenclature, *Phaeocollybia*, chanterelles, PNW mushrooms, and was co-author on major phylogenetic studies.

Lorelei at times seemed to have boundless energy and a joie de vivre. Always cheerful and with a bouncy lilt to her voice. In 2005 she was awarded an MSA Fellowship for researchers in mid careers. She also was immersed in conducting field studies in Oregon with Ron Exeter, together and separately leading to joint publications. She loved nature, exploring the dripping wet west coast rainforests decked out in colourful rain gear.

Ron Exeter and Lorelei in Oregon survey plots sampling chanterelles (Ron) and *Phaeocollybia* (Lorelei)

At home she and Todd maintained a menagerie of cats and dog, and she placed many bird feeders and fed the gang of racoons that appeared regularly at her patio windows. Lorelei's cancer returned in 2012 and being a scientist herself, she began an extended series of treatments including experimental drugs, radiation, and immunotherapy, allowing the medical researchers to test several risky therapies. Shortly after the initial second diagnosis, she began relying more upon Shaun Pennycook to take on additional duties, including copy-editing so that she could concentrate most of her energies on her exceptional production-editing skills. She kept a group of her "specials" up to date via email on all the ups and downs and treatments in a light, humorous, almost suspense

novel dialogue with irony and cheer. One of her greatest joys was the birth of her grandson, Todd Porter Norvell (aka The GREAT grandkid!!), mentioned in many such emails with attached photos.

Lorelei and David Arora at a foray, about 1999 (photo by S.A. Redhead)

Lorelei and Paul Stamets at his business, Fungi Perfecti, in Olympia, WA in 1994 (photo by S.A. Rehead)

Ten years after the cancer's return, Lorelei was still managing the 2022 **Mycotaxon** but with failing energy levels leading to the delayed publication of volume 137(3) in March 2023, and to preliminary work on the current volume 137(4), posthumously completed by Shaun, with Noni Korf, eldest daughter of Richard P. Korf, taking

Lorelei and Shaun Pennycook at Edinburgh IMC 2010

over the production-editing role. Lorelei remained upbeat in an e-mail to Shaun and lengthy phone call with Scott only 2 days before her death. She often said she was born with a happy gene. She never gave up, as evident in an email in 2022, "I have two more issues to complete, after which I return to *Phaeocollybia* full time. (that's the theory anyway!)."

Lorelei's publications, both scientific and popular, are too numerous to list here and will be catalogued elsewhere. However, several are notable for their impact:

Norvell, L.L. 1995. Loving the chanterelle to death? The ten-year Oregon Chanterelle Project. **McIlvainea** 12(1): 6–26. [This was the culmination of a survey conducted by the OMS on the effects on commercial harvesting of *Cantharellus formosus*, the Pacific Golden chanterelle.]

Pilz, D., Norvell, L., Danell, E., Molina, R. 2003. **Ecology and Management of Commercially Harvested Chanterelle Mushrooms.** United States, Department of Agriculture, Forest Service Pacific Northwest Research Station General Technical Report PNW-GTR-576. 83 p

Exeter, R., Norvell, L.L., Cazares, E. 2006. *Ramaria* **of the Pacific Northwest.** U.S. Department of the Interior, Bureau of Land Management, Salem District/ WA/PT-06/050-1792. 157 p

Norvell, L.L., Exeter, R. 2008 *Phaeocollybia* **of Pacific Northwest North America,** Salem, OR, U.S. Department of the Interior, Bureau of Land Management, Salem District. 229 p [This is a comprehensive all color guide with keys and full descriptions of 25 species.]

She anticipated her demise and over the past few years downloaded many of her publications onto Research Gate https://www.researchgate.net/profile/ Lorelei-Norvell. Her son Forrest is maintaining her business's website that also contains a wealth of knowledge. http://www.pnw-ms.com/home.html

Lorelei Norvell authored 12 new species of *Phaeocollybia* (later synonymizing 4) and one new species each of edible PNW *Cantharellus* and *Hydnum*. She was co-author of the genus *Chromosera, Hygrophoraceae* subfamily *Hygrophoroideae* and tribe *Chromosereae,* and *Lichenomphalia* subg. *Protolichenomphalia*. She was honoured by the eponymous agaric genus *Loreleia* in 2002 and will be again soon by the yet unpublished name "*Chromosera loreleiae*".

Lorelei was interviewed regarding her life and career by Leon Shernoff, then editor of **Mushroom, the journal of Wild Mushrooming**, which was published in 4 parts, the first being "The Quest for *Phaeocollybia* (and much more)" and is available on line at https://www.mushroomthejournal.com/lorelei-norvell-interview-part-1/.

She lived a remarkable life and made a major and lasting impact on science and her community.

Lorelei at a conference in the eastern U.S.A., about 2001, wearing her
custom made, gold/stone inlayed *Phaeocollybia* pendant
(photo by S.A. Redhead)
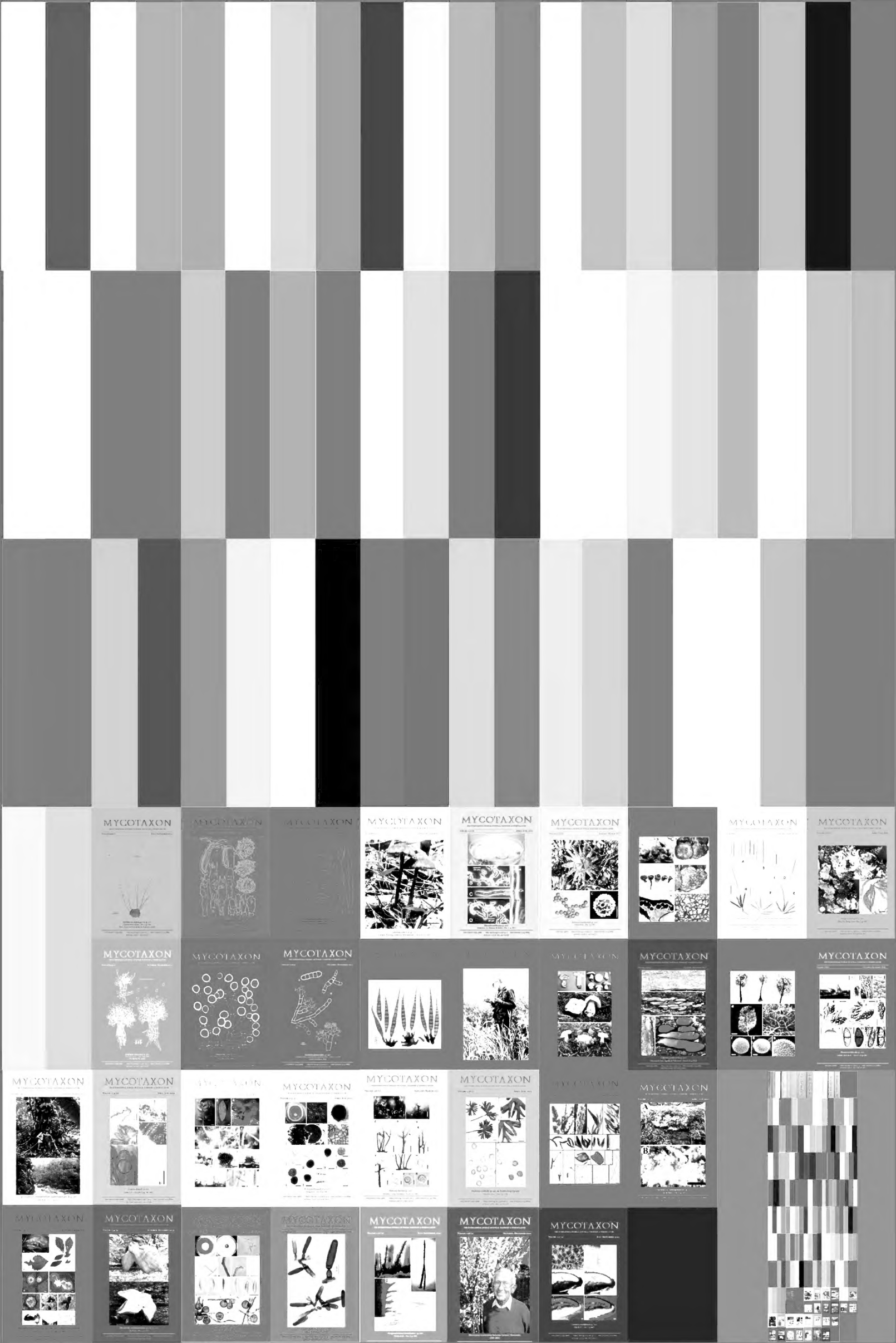
The second 25 years of *Mycotaxon* covers, 1999-2023: volumes 70–137(4)